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(19)(CA) **CANADIAN PATENT** (12)

(54) Single Source Multi-Site Photometric Measurement System

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(30) (US) U.S.A. 782,635 1985/09/30
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(1)   ABSTRACT OF THE DISCLOSURE

A multibeam photometric measuring device includes a
light assembly comprising a single modulated light source
along with a filter assembly for passing only a desired band
of wavelengths and an optical system for suitably focusing
light from the light source. A plurality of light
transmitting means accept light the focused through an
efficient light distribution arrangement and transmit it
individually to each of the plurality of sites disposed on a
multi-well sample plate. A fiber rotor arrangement is
provided for sequentially connecting the collimated light
source to each of the plurality of fibers disposed in the
distributor.

Light passing through the wells of the sample plate is
captured by discrete photodetector cells disposed on a one-
to-one correspondence opposite the wells of the plate. A
reference fiber links light from the light source to a
separate photodetector to provide a reference light signal
with which the sensed photodetector signals are compared.
The sensed signals are suitably multiplexed and processed by
special circuitry which allows control over the accuracy with
which the signals are processed on the basis of their
instantaneous strengths as well as reference readings taken
during system initialization.

Enhancement of color distribution resulting from
chromogenic reactions is provided by automatic agitation of
the reacting agents within the sample wells before a reading

is taken at each of the sampling intervals of a kinetic

measurement sequence.

This invention generally relates to photometric
devices for performing densitometric measurements on
translucent sites. More particularly, this invention
relates to an automatic scanning device using end-
point and/or kinetic measurement techniques for
measuring the optical density of a plurality of
translucent samples in order to analyze their
contents.

A variety of techniques and devices are
commercially available for the detection and
measurement of substances present in fluid or other
translucent samples by determining the light
transmitivity of the sample. In particular, a number
of photometric devices are capable of simultaneously
performing individual assays on a plurality of liquid
or other translucent samples. Such methods generally
handle a multiplicity of samples by using
"microplates" which contain a standard array (8 x 12)
of wells and which are made of an optically

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transparent material. The optical density of the
samples is measured by determining attenuation
undergone by light as it passes through the
translucent samples, contained in the microplate
wells, to conventional photodetecting means.

A widespread use for microplates is in the
enzyme-linked immunosorbent assay (ELISA) technique
which is used for detection and quantitation of an
extensive range of substances and biological cells in
academic research and biotechnology as well as for
clinical testing. In such assays, molecules of a
marker enzyme (such as alkaline phosphatase) are
deposited on the bottom and part of the way up the
sides of each of the wells of a microplate; each well
having been assigned to interact previously, directly
or indirectly, with a sample containing an analyte of
interest. The number of marker enzyme molecules
bound to each well of the plate is a function of the
concentration of analyte in the sample of interest.
Determination of the activity of the bound enzyme,
therefore, permits detection or quantitation of the
analyte.

For determination of fluid-phase enzyme
activity, current techniques for both research and
clinical applications employ kinetic analysis which
involves measurement of the initial rate of enzyme-
catalyzed, chromogenic reactions in the presence of
excess of the enzyme substrate; a procedure which has
several well-known advantages over the alternative
"end-point" analysis method of allowing the enzyme to
react with a chromogenic substrate for a fixed period
of time and then making a single optical density
measurement after quenching the enzymes. In kinetic
analysis, multiple readings are made within the
initial (typically linear) reaction period and the

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intervals between readings are necessarily short
(typically less than 30 seconds). By using kinetic
analysis, the introduction of errors caused by
(a) differences in initial optical density and/or
(b) loss of independence from substrate
concentration, is substantially avoided.

Currently available automated optical density
measurement instruments for microplates typically
function by mechanically moving either the multi-well
microplate or the optical components themselves in
order to successively perform assays of samples
located at the plurality of individual assay sites.
This requirement places a severe restraint on the
time required to actually measure the transmittance
in all wells of the microplate, thereby making large
scale kinetic analysis assay applications impractical
due to the extended sampling times. In part because
of this, "end-point" analysis is employed for ELISA
protocols read by current instruments.

A measurement system which is capable of reading
a plurality of assay sites in sequence without
relative physical movement of the microplate and the
optical components is disclosed in Wertz et al. U.S.
Patent No. 4,408,534 which discloses the use of fiber
optic transmission means with a single light source
sequentially coupled to a plurality of optical fibers
which transmit light to the measurement sites.
However, the apparatus described in the Wertz patent
uses a highly inefficient system for coupling light
from the light source into the optical fibers, which
in turn leads to a variety of potential problems for
kinetic measurements of enzyme activity. For
example, the Wertz apparatus requires a high power
light source and the increased light intensity can
adversely affect the chemical reactions in the assay

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sites by increasing the operating temperature of the
measurement system non-homogeneously and hence
altering the rates of reaction in different wells to
a different extent. In addition, such systems are
unduly complex because of the wide fluctuation in
signal levels generated as a result of the reception
of light at the photodetectors after'it has passed
through the sample sites; this prevents efficient
utilization of the overall dynamic range of
amplification for the signal amplifiers of the
measurement system.

Another limitation of conventional microplate
reading devices is their inability to make useful
quantitative measurements for ELISA protocols
performed in filter-bottom microplates. A principal
problem is that the individual wells in such plates
vary considerably in their initial optical density
relative to air thereby introducing considerable
error when end-point measurements are taken. Kinetic
analysis, on the other hand, is not affected by this
type of problem.

Another major problem associated with
conventional microplate reading devices, when used
for assaying chromogenic reactions kinetically, is
that they are subject to errors arising from erratic
redistribution of the colored product as a result of
phase separation and/or uncontrolled bulk movement of
the aqueous phase of the sample during kinetic
analysis. More specifically, in the case of ELISA
protocols, where the enzyme is bound to the plastic
surface of the microplate wells (on the bottom and/or
part way up the sides), the bound enzyme interacts
with an unstirred aqueous phase layer which typically
causes localized phase separation of the colored
product of the enzyme reaction due to its high local

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concentration. This separation introduces an unquantifiable error and a degree of non-linearity into such kinetic measurements. Even in cases where the colored product remains in true solution, erratic bulk movement of the aqueous phase leads to uneven redistribution of the concentrated product and hence to an unquantifiable error.

It is a primary object of this invention to provide an automatic photometric device for multi-site kinetic and/or end-point densitometric assays, which is capable of accurately acquiring a large number of measurements in a relatively short period of time.

Another important object of this invention is to provide such a photometric measurement device which uses a single relatively low power light source leading to reduced ambient operating temperature and extended life.

Another object is to provide such a photometric device with convenient control over the accuracy with which the sensed signals for this measurement system are processed.

It is a related object of this invention to provide a photometric device of the above kind which does not require relative mechanical movement of the microplate and the optical components of the system in order to perform successive multi-site assays.

Another important object of this invention is to provide such a photometric device which, by virtue of its ability to perform kinetic analysis, is able to make quantitative measurements of enzyme activity in filter-bottom microplates.

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Another important object of this invention is to provide such a photometric device which provides significantly enhanced color distribution in the assay of chromogenic reactions and is not subject to measurement errors arising from nonuniform distribution of the colored product in such chemical reactions.

It is also a related object of this invention to provide a measurement device of the above kind which is efficient, economical and convenient to use.

These and other objects are achieved according to the system of this invention by providing a photometric kinetic measurement device for analyzing samples contained in a plurality of sample sites disposed on a multi-site assay plate, the device comprising: a light source for providing light to one or more of said sample sites; photodetector means for receiving light transmitted through each of said one or more sample sites and for providing a signal in response to said light received wherein the light vertically passes through the sample; means for imparting oscillatory movement to said multi-site assay plate for a pre-determined time before each of a series of readings is performed on a sample site in order to agitate the sample therein; and a means for kinetic analysis of the signal of the photodetector means.

The present invention also provides a multibeam photometric measurement device for measuring optical density of samples contained in a plurality of sample sites disposed on a multi-site assay plate, said device comprising: a single light source adapted through coupling means to be selectively applied, according to a predetermined sequence, through selected ones of a plurality of light transmitting means to selected ones of said samples sites; a plurality of photodetector means disposed on a detector board and positioned in relation to said assay plate in such a way that light transmitted through each of said sites is received by a corresponding photodetector; means for simultaneously imparting mixing movement to said samples

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comprising means for imparting movement to said assay plate for a predetermined time before a reading is performed on a sample site; and means for analyzing the output of said photodetector means to determine and indicate the optical density of said sites.

The present invention also provides a photometric measurement device for measuring optical density of samples contained in a plurality of sample sites disposed on a multi-site assay plate, said device comprising; a light source for applying light to selected ones of said sample sites; photodetector means positioned in relation to said assay plate in such a way that light transmitted through each of said sites is received by said photodetector; means for simultaneously imparting mixing movement to said samples comprising means for imparting movement to said assay plate for a predetermined time before a reading is performed on a sample site; and means for analyzing the output of said photodetector means to determine and indicate the optical density of said sites, said means for analyzing comprising: means for sequentially selecting the analog output signals for desired photodetectors for analysis; means for converting said analog signals to their corresponding digital values; means for amplifying said analog signals in such a way as to utilize a substantial portion of the dynamic range of said analog to digital conversion means; means for storing precalculated values corresponding to said digitized values, accepting said digitized values and retrieving the corresponding stored precalculated value in response to each of said digital values; and means for processing said retrieved values in order to calculate the optical density of said samples.

The present invention also provides a Photometric measurement device for measuring optical density of samples contained in a plurality of sample sites disposed on a multi-site assay plate, said device comprising: a light source for applying light to selected ones of said sample sites; photodetector means positioned in relation to said assay plate in such a way that light transmitted through

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each of said sites is received by said photodetector; means
for simultaneously imparting mixing movement to said samples
comprising means for imparting movement to said assay plate
for a predetermined time before a reading is performed on a
sample site; and means for analyzing the output of said
photodetector means to determine and indicate the optical
density of said sites, wherein said photodetectors are
divided into blocks, each consisting of a plurality of
photodetectors, in such a way that photodetectors in said
blocks are spaced substantially apart on said detector
board, and the outputs of the photodetectors of each said
block are connected together to yield a common output
signal, whereby the output of a selected photodetector can
be analyzed by selecting the common output signal for the
block in which the photodetector lies.

In a further aspect, the present invention provides a
method for measuring the transmittance of a plurality of
sample sites on a multi-site assay plate comprising the
steps of selectively focusing light from a single light
source through a plurality of light transmitting means upon
selected ones of said sample sites, for each sample site,
measuring , at a succession of time intervals, the intensity
of light passing through the reacting sample in the sample
site by using photodetecting means, oscillating said assay
plate for a first fixed time duration in order to agitate
the samples contained therein and allowing the agitated
samples to settle for a second fixed time duration before
performing each of said successive measurements, measuring
the intensity of light when coupled directly from said light
transmitting means to said photodetecting means, measuring
the intensity of light when coupled from said light
transmitting means to said photodetecting means, through the
sample site when it contains no reacting sample, using said
measurements to calculate the optical density of said sample
sites.

In a further aspect, the present invention provides a
method for taking measurements of the transmittance of a
plurality of sample sites on a multi-site assay support,

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comprising the steps of measuring the photosignal generated
by light shining through an airspace in the absence of a
sample to give a first air transmission photosignal value;
measuring the photosignal generated by light shining through
an airspace in the absence of said support to give a second
air transmission photosignal value; adjusting said
relationship to compensate for a difference between said
first air photosignal value and said second air photosignal
value.

In a still further aspect, the present invention
provides a method for determining a colormetric assay
utilizing a multibeam photometer having a photodetector
means positioned in relation to a multi-site solid such that
light is received at a plurality of different sites on the
surface of said photodetector means, said method comprising
the following steps:

measuring the photosignal generated by light shining
through an airspace in the absence of a sample support to
give a first air transmission photosignal value; measuring
the photosignal generated by light shining through an
airspace in the absence of said support to give a second air
transmission photosignal value; adjusting said relationship
to compensate for a difference between said first air
photosignal value and said second air photosignal value.

In a further aspect, the present invention provides a
method for measuring the transmittance of a plurality of
samples contained in a plurality of sample sites on a multi-
site assay plate while said assay plate is in a device for
carrying our said measuring, said method comprising the
steps of:

inserting said assay plate into said device; imparting
mixing movement to said samples by imparting movement to
said assay plate for a first fixed time duration; providing
light to one or more of said sample sites; for each sample
site, measuring the intensity of light passing through the
sample in the sample site; and removing said assay plate
from said device.

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Other objects, characteristics and advantages of the present invention will be apparent from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a simplified schematic representation of a photometric reading device according to the system of this invention;

Fig. 2 is an illustration of the fiver distributor showing the various designated positions utilized to couple the light source to each of the plurality of optic fibers;

Fig. 3 is an illustration of the disposition of the various optical fibers within a fiber manifold associated with the ample assay sites;

Fig. 4 is a simplified block diagram of the processing circuitry according to this invention;

Fig. 5 is a flow chart representing the general sequence of operations involved in a typical sequential scan of the sample plate according to the illustrative measurement system;

Fig. 6 is a flow diagram of the operating sequence undergone by the system as part of the air calibration phase;

Fig. 7 is a flow chart representing the sequence of operations involved in performing the read phase on a sample tray.

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FIG. 8 is a perspective view of a preferred embodiment of a multibeam photometer according to the present invention;

FIG. 9 is a side elevation cutaway view of a microplate well inserted between the LED array and photodetector of the photometer of FIG. 8;

FIG. 10 is a block diagram of the electronic control circuitry for the photometer of FIG. 8;

FIGS. 11A and 11B are circuit diagrams of a portion of the control circuitry of FIG. 10;

FIG. 12 is a timing diagram for portions of the circuit of FIGS. 11A and 11B; and

FIG. 13 is a flow chart of a computer program for controlling the control circuit of FIG. 10.

While the invention will be described in connection with certain preferred embodiments, it will be understood that it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalent arrangements as may be included within the spirit and scope of the invention as defined by the appended claims.

Referring now to the drawings, FIG. 1 is a simplified schematic representation of an exemplary kinetic measurement system 10 embodying the present invention. The system 10 includes a light assembly 12 capable of providing a finely focused reflected beam of light for coupling to the test or assay sites. The light assembly 12 comprises a single source of uniform, reflected and focused light 14 which, in the preferred embodiment, includes a

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tungsten-quartz bulb emitting light in a wavelength
range of about 300 to 900 nanometers. The output of
the light source 14 is passed through a chopper 16
which modulates the light at a fixed frequency which
in the preferred embodiment is 800 hertz.

The modulated light from the chopper 16 is
collimated through a lens 18 onto a filter wheel 19
which extracts light of a selected wavelength and
bandwidth which corresponds to the wavelength at
which the test samples exhibit light absorbency. The
filtered light is passed through a lens 20 which
receives the collimated light and focuses it onto a
precise point 22 from which the light can be coupled
to selected fibers which conduct the light to the
plurality of assay sites.

The kinetic measurement system 10 utilizes a
highly efficient and structurally simple light
coupling and transmitting mechanism. As shown in
FIG. 1, the collimated light exiting the light
assembly 12 is directed to a light coupling
arrangement which includes a cylindrical rotor 24
which is capable of being rotatably positioned
accurately about its axis. The rotor 24 includes an
optical fiber 26 having an input end 28 located at
the center of the rotor 24 and coincident with the
focal point 22 of the light output from the light
assembly 12. The output end 30 of the fiber 26 is
located near the periphery of the fiber rotor 24 so
that as the rotor rotates the input end 28 of the
fiber remains stationary with respect to the light
assembly 12 while the output end 30 moves around a
circular path. The rotor 24 thus permits efficient
coupling of light from the light assembly 12 into
selected ones of a plurality of light transmitting
means which carry the light to corresponding assay
sites.

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The light output from the fiber 26 in the
rotor 24 is received by a fiber optic distributor 32
containing a multiplicity of optical fibers 34 having
their input ends arranged in a circular array. This
circular array of fiber ends has the same radius as
the location of the output end of the fiber 26 in the
rotor 24 so that as the rotor 24 is indexed about its
axis, the output end of the fiber 26 is brought into
alignment with successive fibers 34 in the
distributor 32. On the output side of the
distributor 32, the fibers 34 are brought together to
form a fiber bundle 36 which leads to a fiber
manifold 38 which aligns each of the fibers 34 with
one of the assay sites (to be described below).

The rotor 24 is indexed by means of a suitable
stepper drive or other appropriate controllable
displacement means so that it sequentially directs
the collimated light from the focal point 22 into
different fibers 34 of the bundle 36.

The use of the optical fiber coupling
arrangement to couple light from the light
assembly 12 to individual optical fibers 34 within
the distributor 32 constitutes a significant
advantage because of the efficiency of transmission
associated with the mechanism. More specifically,
the optical fiber 26 ensures that substantially all
the collimated light from the light source 14 is
coupled into a selected optical fiber 34 without any
significant diffusion at the point of coupling at
either end of the coupling fiber 26. Thus, light
from the source 14 is coupled through the rotor 24
only into a selected one of the optical fibers 34
with minimal undesired coupling of light into optical
fibers adjacent to the selected fiber. This highly
efficient coupling allows the use of a single light

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source with relatively low power capacity since a high percentage of light emanating from the source is transmitted to the site of measurement.

The illustrated light source in FIG. 1 is a convenient and preferred form; however any light source or a plurality of light sources providing light of equal intensity and having the desired range of wavelength can be used. In this regard, an alternative form of photometric measurement, which is based upon a sequentially operated multibeam light source, will be described in detail below in connection with FIGS. 8-13.

At the site of measurement, light emanating from any of the optical fibers 34 located within the fiber manifold 38 is directed to a corresponding assay site on a sample plate 40 through an array of lenses 42 disposed between the manifold 38 and the sample plate 40. The sample plate 40 may be a conventional microplate having a series of wells, usually an array of 96 wells arranged in 8 rows having 12 wells each. The sample plate is preferably mounted in an area where the ambient temperature is regulated, e.g., by means of a fan, so as to present isothermal conditions about the sample plate. This is important in minimizing inaccuracies from varying rates of reaction occurring at different sample sites as a result of a temperature gradient about the sample plate.

An array of photodetectors 44 is provided on a detector board 46 in the form of a matrix conforming to the positions of the wells located on the sample plate 40. A second lens array 48 is positioned beneath the sample tray 40 and serves as means for focusing light from each well of the sample plate 40, after the light has passed through the well. The

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lens arrays 42 and 48 are of the conventional type
and include apertures (not shown) which function to
direct the light towards the individual lenses and
minimize diffusion of light into adjoining lenses.
The sample plate 40, or at least the bottom of each
well in the sample plate, is translucent or
transparent so that light coupled to a particular
optical fiber within the fiber manifold 38 and then
collimated onto a corresponding sample well
penetrates the well and its contents, passes through
the corresponding focusing lens disposed within the
lens array 48, and reaches the corresponding photo-
cell 44 located immediately below the sample well.

The photodetector 44 senses the intensity of the
light passing through the corresponding sample well
and produces an electrical output signal proportional
to the intensity of light incident on its surface.
Each of the photodetectors 44 provided on the
detector board 46 functions in a similar manner and
provides a signal which is proportional to the
varying intensity of the light impinging thereon.
This varying intensity is caused by the varying
transmittance offered by the sample as a chemical
reaction progresses therein and alters its
constituency. The resulting electrical signals from
the photodetectors 44 are fed to an analysis and
indication system 50 which processes the received
signals and provides an external indication of the
transmittance or optical density of each of the
samples contained within the multiple wells of the
sample plate 40.

The measurement system of Fig. 1 also includes a
single reference optical fiber 52 positioned adjacent
to the focal point 22 so that the fiber 52 receives
light continuously whenever the light source 14 is

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energized, i.e., whenever any of the sample sites is
being tested. The light emanating from the output
end of the reference fiber 52 is coupled through air
or an empty sample well to a separate
photodetector 45 on the detector board 46, and
provides a reference photodetector signal whose
significance will be described in detail below.

The optic fiber distributor 32 also has an
optical fiber designated as the "home" fiber which
serves as a reference for determining the current
position of the distributor 32 relative to the
coupling rotor 24. The function of the home fiber
will also be described in more detail below.

In order to promote uniform distribution of the
color resulting from chromogenic reactions in the
samples being measured, the measurement system of
FIG. 1 is provided with means for agitating the
chemical solutions contained within the plurality of
wells of the sample plate 40. More specifically, as
shown in FIG. 1, the sample plate 40 is attached to
an agitating mechanism 54 which oscillates the tray
in a manner that brings about a thorough mixing of
the chemical solutions contained within the sample
wells.

A common problem with conventional microplate
readers, if used for kinetic analysis of ELISA, is
that distribution of the color produced as a
chromogenic reaction progresses may be uneven across
the width of a sample well. Since kinetic optical
density measurements generally involve the
transmission of light through the central portion of
a sample well, uneven distribution of color (e.g.,
due to color development on the walls or corners of a
sample well) can severely distort the accuracy of the
transmittance readings. Non-homogeneous distribution

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of color in the samples can also produce significant variations in the measured values from one cell to another, even if similar chemical reactions are occurring in the cells, thereby making the transmittance measurements unreliable.

Although certain previous measuring techniques have suggested manual shaking of the microplate prior to reading, random diffusion of the color as the reaction progresses can introduce unpredictable, non-reproducible results into the otherwise generally linear variation of the kinetically measured optical density with respect to time; that is, the transmittance of the sample becomes dependent on the progressively non-homogeneous color distribution in the sample. This non-reproducibility cannot be overcome by shaking the microplate at the beginning of a kinetic reading cycle because, unlike conventional end-point measurement where the reaction is quenched prior to the measurement phase, kinetic reading involves measurement of optical densities while the reaction progresses unquenched. Because the color development continues as the reaction progresses, initial shaking, even though it provides a certain degree of linearity, is insufficient to produce a uniform distribution of color throughout the reacting solution and causes low precision measurements.

It has been found that agitation of the reaction agents immediately prior to each of the multiple readings taken within a kinetic reading cycle, in accordance with the present invention, significantly improves the measurement accuracy by ensuring a homogeneous distribution of the color product throughout the sample as the chemical reaction progresses. In the particular example illustrated in

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FIG. 1, such homogeneous color distribution is achieved by oscillating the entire microplate before each reading is taken. This oscillatory movement agitates the reacting agents within each of the sample wells so as to prevent any localized separation of the colored products of the enzyme reactions. In this manner variation in the transmittance of the samples within the sample wells is maintained substantially linear with respect to time over an entire kinetic reading cycle.

In the particular embodiment shown in FIG. 1, the sample plate 40 is connected to an agitation mechanism 54 which comprises a motorized arrangement capable of imparting gentle oscillatory motion to the sample plate at a desired frequency of oscillation. According to a preferred embodiment, the agitation mechanism 54 oscillates the sample plate with the same reversible drive motor generally used to control the position of the sample plate, by energizing the motor repeatedly in opposite directions at the desired rate of oscillation. Satisfactory results are obtained by oscillating the sample plate over a distance of about 1/16th of an inch at a frequency of about 20 Hz. The measurement system is programmed to oscillate the sample plate before each reading. The oscillation is followed by a short time delay before the start of the reading phase to allow the reacting solutions within the sample wells to settle. The delay interval prevents erroneous readings due to reflection and/or refraction effects arising from ripples caused by the agitation process. Typical time periods are 3 seconds for the agitation phase and 1 second for the delay interval.

The agitation need not be effected by mechanical means, as long as the objective of achieving

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homogeneous color distribution effect is realized.
Other suitable agitation means, such as "ultrasonic"
vibration inducement, may be used, depending upon the
desired efficiency and the constituency of the
reacting agents. Further, the oscillatory movement
of the sample plate need not be limited to lateral or
rotational movements parallel to the plane of the
sample plate; vertical oscillation (up-and-down
movement) can also be used satisfactorily as long as
spilling of test samples can be avoided.

Referring next to FIG. 2, the fiber manifold 38
is shown in more detail, including the disposition of
the various optical fibers across the lower surface
of the manifold. It will be noted that this
particular embodiment of this invention is designed
for the sequential assaying of samples contained in a
conventional microplate which has 96 test sites or
wells arranged in 12 x 8 matrix. Accordingly, as
shown in FIG. 2, the fiber manifold 38 has 96 fibers
arranged in 8 rows A, B, C, D, E, F, G and H, each
row consisting of 12 fibers. For instance, the row A
has 12 fiber ends A_1, A_2, A_3 . . . A_{12}.

Also located in the manifold 38 are the output
ends of the reference fiber 52 and the "home"
fiber. The arrangement of the 12 rows A-H and the
separation between adjacent fibers corresponds
exactly to the arrangement of the sample test sites
in the sample plate used for the assay. Thus, light
transmitted through a particular fiber is collimated
directly onto the corresponding sample well and
passes through to the corresponding photodetector,
thereby generating an electrical signal having a
magnitude proportional to the intensity of the light
impinging on the photodetector.

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FIG. 3 is a schematic representation of the fiber distributor arrangement showing the circular array of the input ends of the various fibers located in the fiber manifold 38. As shown, the distributor 32 carries the input ends of each of the 98 optical fibers disposed within the fiber manifold. These 98 fibers are arranged in a circular array around the distributor 32. The input end of the "home" fiber is located in a position preceding the first row A of optical fibers. Three opaque spots are located between the "home" fiber and the first fiber A_1 which corresponds to the first sample site to be assayed; these opaque spots serve to reference the start of a new assay sequence, as will be described below.

Referring now to FIG. 4 there is shown a block diagram of the processing circuitry used for the analysis of the signals generated by the photodetectors 44. In the illustrative embodiment, the detector board 46 actually has 98 photodetectors 44, 96 of which correspond to the 96 sample sites of the sample plate 40 (FIG. 1), one of which receives light focused upon it from the reference fiber 52, and one of which receives light from the "home" reference fiber. It will be apparent that only a single reference photodetector is required if a single fiber is used to perform both the light and home reference functions. The signals generated by the 96 photodetectors for the sample sites are converted to a corresponding voltage form by a current-to-voltage converter 110. These signals are then passed through a band limiting low pass filter 111 and subsequently to a multiplexer 112 which functions to controllably select desired ones of the 96 signals for further analysis according to a pre-programmed sequence.

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The signals generated in response to light from
the light reference fiber 52 and the "home" fiber are
converted into their equivalent voltage form by
current-to-voltage conversion units 113 and 114 and
then passed through band limiting low pass
filters 115 and 116, respectively. The outputs of
the multiplexer 112 and the two low pass filters 115
and 116 are fed to a second multiplexer 118 which
functions to controllably select one of the three
signals at its input for further analysis.

The "home" reference fiber provides a convenient
means for locating the angular position of the
fiber 26 in the rotor 24 relative to the fiber
distributor 32. More specifically, the "home"
reference fiber serves as a means for ensuring that
the rotor 24 is positioned correctly so that the
light output from the coupling fiber 26 is directed
to the proper optical fiber in the distributor 32 to
begin a sequential assaying process. Since any light
entering the "home" reference fiber is transmitted
directly to a dedicated "home" photodetector 44, the
existence of a peak signal at the output of the
"home" photodetector is an indication that the
rotor 24 is positioned at a so-called "home" position
where the coupling fiber 26 is aligned with the
"home" reference fiber.

Any time a new assay is to be started, the
measurement system first positions the rotor 24 at
its "home" position. In order to accomplish this,
the rotor 24 is rotated while monitoring the output
of the "home" photodetector, until a peak output
signal from that photodetector is detected. Since
the position of the "home" reference fiber relative
to the first optical fiber A_1 is a known factor (in
this case, separated by the three opaque spots),

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subsequent alignment of the coupling fiber 26 with
the fiber A_1 can be conveniently accomplished by
stepping the rotor 24 through three consecutive
positions.

The provision of the light reference fiber 52
serves as a calibration means by which only the
differences in sensed light intensity from one
reading to another may be utilized for analysis of
sample transmittance, thereby eliminating the effects
of variations in the light output of the light
source, system drifts and the like. More
specifically, each time an assay is performed, the
signal produced by the light reference fiber is
measured before the start of the reading cycle. Then
all subsequent signals produced by the photodetectors
for the test sites are scaled relative to the signal
initially produced by the light reference fiber. In
this way, only actual changes in the transmittance of
the liquid samples as a result of the chemical
reactions occurring at the sample site are
measured. Any localized differences in intensity
such as those resulting from fluctuations in light
intensity from the light source over the course of
its lifetime, or premature readings taken before the
light source or other system parameters have had a
chance to stabilize, are disregarded.

Since the "home" reference fiber is coupled
directly to the corresponding photodetector, it can
also be conveniently used as a reference light
reading source and can effectively provide all
functions performed by the light reference fiber as
described earlier, in addition to providing a
reference for determining the position of the fiber
rotor 24 relative to the fiber distributor 32.

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Returning now to FIG. 4, the signal selected by
the multiplexer 118 is passed through a conventional
bandpass filter 119 with a reasonably large bandwidth
centered about the frequency at which the chopper 16
modulates the light passing through it (e.g.,
800 hertz). The bandwidth of the bandpass filter 119
is selected to be sufficiently large to accommodate
the fluctuation in signal settling time that occurs
as a result of rapid readings being performed on the
sample plate. The band-limited output of the
filter 119 is then fed to a variable gain amplifier
120 whose function will be described below.

The output of the amplifier 120 is fed to a two-
way switch 122 which in its normally closed position
provides a direct electrical connection from the
amplifier 120 to the subsequent portion of the
processing circuitry. When the two-way switch 122 is
open, the preceding portion of the measurement system
is cut off and the switch 122 serves as a virtual
short circuit for measuring the signal generated only
by the rest of the processing circuitry. Thus, the
switch 122 serves as a means for determining the
"dark current" flowing through the processing
circuitry in the absence of any light passing through
the fiber distributor 32. This dark current
measurement is free from distortions and noise from
sources preceding the two-way switch 122, because the
section of the processing circuitry in which the
current is measured is totally isolated from the rest
of the measurement circuitry. The significance of
taking such readings while the circuit is isolated
from the light source will be described below.

From the two-way switch 122, the output signal
is passed through a high pass filter 124 which in
combination with the low pass filters 111, 115 and

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116 provides secondary bandpass characteristics to
the processing circuitry. The output of the high
pass filter 124 is fed through a phase detector 126
which in conjunction with a reference input from the
light source chopper 16 and a low pass filter 128
functions to extract a direct current signal
corresponding to the alternating current resulting
from the modulating effect of the chopper 16. More
specifically, the phase detector 126 effectively
inverts the negative portions of the alternating
output signals from the detector board 46 on the
basis of the timing input provided from the chopper
16. The average value of the resulting signal
represents the DC equivalent of the alternating
signal. To extract this average value, the output of
the phase detector 126 is fed through a low pass
filter 128.

    The output signal from the low pass filter 128
is fed to a second variable gain amplifier 130 which
is a high precision amplifier providing a series of
well defined gain settings. The operation of the
variable gain amplifier 130 in conjunction with the
first variable gain amplifier 120 in providing
controllable accuracy and increased dynamic range for
the processing circuitry of this invention will be
described in detail below. The output of the
amplifier 130 is passed through a low pass filter 132
and then on to an analog-to-digital converter 134
which functions to translate the amplified analog
signal into its corresponding digital form. The
digital signals produced by the analog-to-digital
converter 134 are then fed to a conventional digital
microprocessor system which performs a series of
mathematical calculations and comparisons required to
determine the optical density of the samples on the

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basis of a predefined algorithm. The same digital
microprocessor system is also used to regulate the
sequential scanning of the plurality of test sites,
the different multiplexing arrangements, and all
related processing circuit functions.

According to a feature of this invention, the
measurement system is made more efficient and
economical by effectively augmenting the dynamic
range of the analog-to-digital converter used to
represent the analog signals defining the processed
values of the various signals produced by the
photodetectors. The variable gain amplifiers 120 and
130 function to adjust the extent to which the
processed signals are amplified in such a way that
even when the signals vary over a wide range of
amplitudes, a major portion of the quantization
levels of the analog-to-digital converter is utilized
without exceeding its original dynamic range. The
actual operation of the amplifiers will be clarified
by considering the case where the ADC 134 in FIG. 1
having a 12-bit capacity so that the total number of
quantized output levels is 4096, ranging from 0-
4095. As the wavelength of light extracted (by the
filter wheel 18) from the light emanating from the
light source changes, the intensity of the resulting
light also varies. This, in combination with
variations in light transmission characteristics from
one optical fiber to another and the frequency
response characteristics of the photodetectors
themselves, as well as changes in transmittance due
to chemical changes in the samples, can produce
signals whose amplitudes vary over a significant
range.

In order to ensure that the signal with the
largest possible amplitude does not result in an

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output value that exceeds the range of the ADC, it is
conventional to use a low gain amplifier. However,
the disadvantage associated with such an arrangement
is that the low gain amplifier produces a low level
digital output in cases where the intensity of light
received at the photodetectors produces a relatively
weak sensed signal, thereby utilizing the dynamic
range of the ADC very inefficiently. Conventional
recourse in such situations has been to upgrade the
system by using an ADC with a higher bit-processing
capacity, which significantly adds to the overall
system cost.

According to this invention, the variable gain
amplifiers 120 and 130 initially process the incoming
signal at very low gain settings. The resulting
digital output is then compared with the maximum
possible value of the ADC output in order to
determine the maximum possible gain to which the
incoming signal may be subjected without exceeding
the maximum digital output value of the ADC. For
instance, if an input signal produces an ADC output
of about 50 counts at the initial gain setting (which
are normally 1), the system compares this count value
to the maximum count value possible with the ADC
which, in the case of the 12-bit ADC, is 4095 and
determines the extent to which the signal may be
safely amplified so that the digital output falls
within the maximum count value.

A safety margin of about 10% is built into this
dynamic ranging process by performing the above
comparison not on the basis of the maximum output
value of the ADC but instead by comparison with a
value that is roughly 90% of the maximum output. For
the 12-bit ADC, the actual count of 50 would hence be
divided into a safety-adjusted maximum output of 3686

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counts to give a desired amplification factor of about 74. Subsequent to this determination, the gain settings of the variable gain amplifiers are adjusted to closely approximate the desired gain factor. The above arrangement thus allows maximum utilization of the dynamic range of the measurement system, regardless of variations in the relative strengths of the signals generated by the photodetectors.

According to this invention, the gain $G1$ of the first variable gain amplifier 120 is adjusted on the basis of the signal derived from light being transmitted through air, whereas the gain $G2$ of second variable gain amplifier 130 is adjusted on the basis of the signals derived from the sample site photodetectors. The actual sequence of operations involved in the adjustment of gain settings for the variable gain amplifiers as well as the overall method of operation of the measurement system will now be described below with reference to FIG. 5.

FIG. 5 is a flow diagram of the general sequence of operations involved in a typical sequential scan according to the system of this invention. The sequence begins at step 150 where the various system variables, such as the number of readings to be made within the kinetic reading cycle, duration of agitation, the duration of the delay following the agitation phase, etc., are initialized. At step 152, the air calibration phase is carried out by performing a reading upon each of the sample wells through air. Measurements made during the air calibration phase are performed with a unity gain setting $G2$ for the second variable gain amplifier 130 and the gain $G1$ of the first variable gain amplifier 120 is optimized for the maximum dynamic range of the analog-to-digital converter. The various steps and

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measurements undergone as part of the air calibration phase 152 will be described in detail below.

The air calibration phase 152 is followed by step 154 where a kinetic reading cycle is initiated upon the basis of the initialization data provided to the measurement system as part of step 150. The kinetic reading cycle includes the execution of the agitating, delay and reading steps at each of a series of pre-programmed discrete time intervals at which optical density readings are to be taken for the particular samples being measured. It will be apparent that, in the case of end-point analysis, the reading cycle will comprise the execution of the above steps only at a single time interval.

Step 156 is the read phase, which includes a series of three steps beginning with the agitation phase 158 during which the sample plate is vibrated for a pre-defined time interval. Subsequently, at step 160, the settling phase takes place during a pre-defined delay interval in which the oscillation mechanism is dormant and the reaction agents within all the sample wells of the sample are allowed to settle down for a pre-defined time interval before obtaining the actual signal readings. At step 162 the measurement system obtains the transmittance reading on all the wells of the sample plate. This step includes optimization of the gain setting G2 for the second variable gain amplifier 130 while the setting maintaining the gain setting G1 of the first variable gain amplifier 120 at the optimized value determined during air reference. The sequence of events involved in the read step 162 will be described in detail below.

Following the read step 162, a check is made at step 164 to determine whether the system has

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completed the pre-defined kinetic reading cycle. If
the answer at step 164 is no, step 166 continues the
kinetic reading cycle. The read phase 156 is
reiterated by the measurement system until the
agitation phase and the accompanying delay and read
sequences have been performed at each of the
prescribed intervals of the kinetic reading cycle.
If it is found at step 164 that readings have indeed
been performed at all prescribed time intervals, the
measurement system comes to a stop. This marks the
end of the kinetic reading cycle.

The reading cycle has been described above only
with respect to the sequence of operations undergone
by the illustrative photometric measurement divide in
obtaining the various light readings required to
calculate the optical density at the sample sites.
It will be understood that the microprocessor system
which forms part of the analysis and indication
system (FIG. 1) processes the data resulting from the
measurements as the reading cycle proceeds and
initiates computation of optical density values (on
the basis of a pre-defined algorithm as will be
explained below) for those sample sites and time
intervals for which required measurements have been
completed.

The following definitions and symbols will be
used in the ensuing description of the various
operations carried out by the measurement system of
this invention during the air calibration and read
phases.

OD_n: The calculated optical density of a given
sample well (where n varies from 1 through 96 in
order to designate each of the 12 sample wells
positioned along each of the 8 rows A through H of
the microplate).

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W_n: The signal output of a photodetector corresponding to a given sample well containing the reacting sample.

G1: The adjustable gain of the first stage variable gain amplifier 120 (controllable by a set of gain multiplication factors including 1, 2, 4, 8, 16, 32, 64 and 128).

G2: The adjustable gain factor for the second stage variable gain amplifier 130 (adjustable by a set of gain multiplication factors including 1, 10 and 100).

D_n: The dark current reading taken with the two-way switch 122 of FIG. 4 in its open position, for a given sample well. This reading is taken with the first stage gain setting G1 set to 1 and at the same second stage gain setting G2 used to obtain the corresponding W_n signal output.

$W.AIR_n$: The signal reading of a given sample well's air calibration taken with the secondary stage variable gain amplifier at a gain setting G2 = 1.

D_{air}: The dark current reading of an air calibration performed with the secondary stage gain setting G2 = 1.

$L.REF_{air}$: The light reference signal reading taken during an air calibration with the secondary stage gain setting G2 = 1.

$L.REF_{read}$: The light reference signal taken at the initiation of a READ cycle with the secondary stage variable gain setting G2 = 1.

D_{read}: The dark current readings taken at the beginning of a READ cycle.

Referring now to FIG. 6, there is shown a flow chart of the sequence of operations included within the air calibration phase of FIG. 5. At the first step 200, the measurement system positions the rotor

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24 at the reference "home" position by sequentially
displacing the rotor until the existence of a peak
signal is detected at the output of the "home"
reference photodetector. At this stage, the gain
settings for both the first and second stage variable
gain amplifiers 120 and 130 are set to unity.

The next step in the air calibration sequence is
step 202 where the measurement system switches to
either the photodetector for the light reference
fiber or the photodetector for the home reference
fiber in order to measure the light reference signal
$L.REF_{air}$. This reading is measured with the gain G2
of the secondary stage variable gain amplifier set to
unity and the gain G1 of the first stage variable
gain amplifier optimized to provide the largest count
value at the output of the analog-to-digital
converter 134 (according to the safety-adjusted
dynamic ranging procedure described above). Also at
step 202, the measured reference signal value
$L.REF_{air}$ and the optimized first stage gain setting
$G1_{L.air}$ are stored in the memory of the
microprocessor system for later use in the optical
density calculations.

At the succeeding step 204, the rotor is
displaced through a designated number of positions
relative to the "home" position so as to locate the
rotor at a position corresponding to one of the three
opaque spots X_1, X_2 and X_3 on the fiber distributor
32. According to the preferred embodiment, the rotor
is actually displaced by two positions relative to
its home position so that the coupler comes to rest
at a position corresponding to the opaque spot X_2.
In this position, the opaque spot effectively blocks
the coupling of any light from the coupling fiber 26
into any of the fibers in within the fiber manifold

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32 and hence isolates the light source from the photodetectors.

At the succeeding step 206, the dual position dark current switch 122 activated and a dark current reading D_{air} is taken with both the first and second stage gains G1 and G2 set to unity. The dark current reading D_{air} represents the residual current flowing within the portion of the processing circuitry of FIG. 4 following the two-way switch 122. This value is subtracted from the signal reading of every sample well in order to provide a true representation of the transmittance value for the sample well at any designated time. Also at step 206, the measured dark current reading D_{air} is stored in the system memory for later use in calculating the optical density.

At this stage, the measurement system is ready to perform air calibration readings on each of the sample wells. Accordingly, at step 208, the rotor 24 is advanced to the position A_1 corresponding to the first sample well of the microliter plate, and the photodetector corresponding to the sample well A_1 is switched on. At the succeeding step 210, the air calibration signal reading $W.AIR_n$ for the sample well A_1 is taken with the gain G2 of the secondary stage variable gain amplifier set to unity and the gain G1 of the first stage variable gain amplifier optimized to a value $G1_{A1}$; the latter value represents the gain setting which allows the maximum safety-adjusted output from the analog-to-digital converter without exceeding its rated dynamic range. At the end of step 210, the measured signal reading $W.AIR_n$ (in this case $n = A_1$) and the optimized gain setting $G1_{A1}$ are stored in the memory.

At step 212, the microprocessor system checks to determine whether air calibration has been performed

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at each of the 96 sample well sites on the sample microplate. If the answer at step 212 is negative, step 214 advances the rotor to a position corresponding to the succeeding sample well site before reverting to the air calibration step 210. If the answer at step 212 is positive, i.e., air calibration has indeed been performed on all 96 sample well sites, it marks the end of the air calibration sequence at step 216. It will be noted that the entire air calibration sequence is performed with the sample plate in its retracted position, i.e., away from the photodetector board so that light from the fiber manifold 38 is transmitted directly to the photodetectors.

Referring now to FIG. 7, there is shown a flow chart of the sequence of operations undergone by the measurement system while performing the read phase. It should be noted that before actual reading is performed on a a sample plate, the measurement system proceeds through the air calibration phase with the sample plate in its retracted position. Prior to the start of a read phase, the sample plate is moved into its advanced position in preparation for the reading phase.

At the start of a reading operation (step 300 in FIG. 7), the measurement system locates the rotor at the "home" position. For this purpose, the signal from the "home" reference fiber photodetector is tracked by the processing circuitry with both the first and second stage variable gain amplifiers having their gains G1 and G2 set to unity.

Step 302 is then accessed, where a measurement of a light reference signal occurs. More specifically, the measurement system switches to the photodetector corresponding to the light reference

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fiber (or the photodetector corresponding to the home
reference fiber if this fiber is being used to
perform the functions of the light reference fiber),
and a light reference signal $L.REF_{read}$ is taken with
G2 set to unity and G1 optimized to its maximum value
$G1_{L.air}$ according to the dynamic ranging procedure
described above. Also as part of step 302, the
measured $L.REF_{read}$ value is stored in the system
memory for later use during calculations of optical
density.

At the succeeding step 304, the rotor 24 is
displaced through a designated number of positions to
locate it at one of the three opaque spots X_1, X_2 and
X_3 provided on the fiber distributor 32. More
specifically, the rotor is stepped two positions
relative to the home reference fiber so as to be
located at the second opaque spot X_2.

The following step 306 actuates the dual
position switch 122 and measures a series of dark
current readings $D_{air.x}$ with the first stage variable
gain G1 set to unity. A single reading is taken at
each of the possible gain settings G2 (in this case
1, 10 and 100) of the secondary stage variable gain
amplifier. The measured values of $D_{air.x}$ are also
stored within the system memory as part of step 306.

At the succeeding step 308, the rotor is
advanced to the first sample well position A_1. In
addition, the system switches to the photodetector
corresponding to the first sample well A_1 to begin
the actual sequential reading cycle. The succeeding
step 310 activates the agitation mechanism for a pre-
designated time interval T_1 to promote homogeneous
color distribution as described above.

The agitation phase of step 310 is succeeded by
a settling phase at step 312 during which the

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agitation mechanism is deactivated and the system
dwells for a time interval T_2 to allow the agitated
samples to settle down in preparation for performing
transmittance readings upon them. The agitation
phase at step 310 may entail displacement of the
sample from its position between the fiber manifold
and the photodetector board to permit oscillatory
movement of the plate. Accordingly, the settling
phase may actually take place during the time it
takes to reposition the plate from its agitation
position to its reading position. Immediately after
agitation and the subsequent settling of the reacting
samples within the sample plate, optical density
readings are taken.

At step 314, the signal W_{An} for the first sample
well, i.e., A_1, is measured with G1 set to the
corresponding stored gain value $G1_{A1}$ determined as
part of the air calibration (step 210 in FIG. 6).
During this measurement, G2 is initially set to unity
and then optimized to a value that produces the
maximum safety-adjusted output value from the analog-
to-digital converter of FIG. 2. Also, as part of
step 314, the measured signal value W_{A1} is stored
within the system memory for use in calculation of
the optical density for that sample well.

At the succeeding step 316, the microprocessor
system performs a check to determine whether signal
readings have been obtained for all 96 sample
wells. If the answer at step 316 is negative, the
microprocessor system advances the rotor to a
position corresponding to the next sample well. At
the same time, the processing circuitry switches to
monitor the photodetector corresponding to the
selected sample well. The system then reverts to
step 310 and goes through the agitation, settling and

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(1) without any sample and (2) with a sample. All
readings measured by the processing circuitry of the
microprocessor system are adjusted for any offset
voltages generated by the analog-to-digital converter
or other system offsets and drifts by taking into
account the corresponding dark current readings, as
indicated in the above equation for OD_n. For
instance, the signal reading $W.AIR_n$ is normalized for
dark current effect by subtracting from it the value
D_{air} of the corresponding dark current reading.
Similarly, this signal reading W_n is adjusted by
subtracting from it the corresponding dark current
reading D_n.

The second quantity $(L.REF_{read}-D_{read})/(L.REF_{air}-D_{air})$ is a measure of the ratio of the light
reference readings obtained for a given sample well
during the air calibration phase and the read
phase. These two readings are also normalized on the
basis of the corresponding dark current readings.

Finally, the third quantity $(G2_n)$ in the above
equation accounts for the effect of the dynamic
ranging procedure described above, i.e., this
quantity neutralizes the effects of amplification of
the signal readings by the processing circuitry.

The application of the above equation to the
parameters measured as part of the kinetic reading
cycle results in a highly accurate optical density
measurement because the equation takes into account
the effects of system offset voltages as well as
those resulting from localized differences in light
intensity and measurement conditions from one kinetic
reading cycle to another, or from one sample well to
another.

In accordance with a further feature of this
invention, the computation of the logarithms required

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to calculate the optical density readings is
performed by storing all the required logarithmic
values within the microprocessor system in the form
of a look-up table and subsequently using the
digitized output of the processing circuitry as an
index to retrieve the appropriate logarithmic
value. In previous systems, the output signals of
the photodetectors of the detector board have been
fed to a logarithmic amplifier to obtain the
logarithmic values of the output signals. This
technique is subject to a variety of problems and
limitations because of the constant need to adjust
the system for offset gains of the logarithmic
amplifiers. In addition, any temperature drift in
the computing hardware must be accurately tracked and
appropriately compensated to retain the accuracy of
computation. According to the present invention, the
computation of the logarithmic values is made
substantially more accurate and independent of the
system hardware parameters by storing within the
microprocessor system memory all possible logarithmic
values that would be required by the system in order
to compute the optical density readings.

More specifically, the look-up table contains
logarithmic values corresponding to each of the
possible output quantization levels for the analog-
to-digital converter of the system (as shown in
FIG. 4). Considering, for example, the case of a
12-bit analog-to-digital converter, the possible
quantization levels range from 0 to 4095. This means
that there are 4096 different values that a signal
may take after it has been detected, processed and
digitized. The logarithmic value corresponding to
each of these 4096 possible values are stored within
a logarithmic look-up table which is contained within

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the ROM portion of the microprocessor system. The logarithmic look-up table is defined in such a way that the digitized output from the analog-to-digital converter serves as an address or index that points to the corresponding logarithmic value stored within the look-up table.

Because the look-up table is available within the microprocessor system memory, computation of the optical density becomes a simple matter of using the digitized output to extract the corresponding logarithmic value stored within the look-up table, and then performing simple mathematical subtractions.

According to yet another feature of this invention, the process of selecting a particular photodetector from the multiplicity of detectors on the detector board is simplified by use of a tiered parallel addressing scheme for the photodetectors. More specifically, the photodetectors are divided into a selected number of blocks, each block consisting of a plurality of photodetectors. Corresponding photodetectors in the various blocks are connected in a parallel fashion in such a way that if, for instance, the first photodetector in the first block is addressed, the measurement system also automatically addresses the first photodetectors in the remaining blocks.

Considering, for example, the case of a 96-well microplate, the detector board comprises 97 photodetectors (96 for the 96 sample wells and 1 for the "home" and light reference fiber). According to a preferred embodiment of this invention, the 96 sample-well photodetectors are divided into blocks consisting of 16 photodetectors each. The first block consists of the photodetectors A_1-A_4, B_1-B_4, C_1-C_4 and D_1-D_4, the second block consists of the

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photodetectors A_5-A_8, B_5-B_8, C_5-C_8 and D_5-D_8, the third block consists of the photodetectors A_9-A_{12}, B_9-B_{12}, C_9-C_{12} and D_9-D_{12}, fourth block consists of the photodetectors E_1-E_4, F_1-F_4, G_1-G_4 and H_1-H_4, the fifth block consists of the photodetectors E_5-E_8, F_5-F_8, G_5-G_8 and H_5-H_8 and the sixth block consists of the photodetectors E_9-E_{12}, F_9-F_{12}, G_9-G_{12} and H_9-H_{12}. With this block configuration, the first photodetectors in all of the blocks, that is A_1, A_5, A_9, E_1, E_5 and E_9, are connected in parallel, and the rest of the photodetectors are connected in a similar fashion.

The addressing of individual photodetectors is considerably simplified since the same address is applicable to the selection of any one of the six photodetectors. Even though six photodetectors are turned on each time any one of them is to be actually monitored, the detectors within any given block are spaced sufficiently apart on the detector board so that the desired photodetector is not affected by light scattering from the other five photodetectors in that block.

The following description with respect to FIGS. 8-13 relates to a preferred embodiment of a rapid solid state multibeam photometer for multi-site microanalysis which can perform a large number of readings in a short period of time without requiring mechanical movement of the optical components or the microplate or other multi-site assay sample. A number of light sources are arranged so that one light source shines on each of the assay wells or spots. At least one monolithic photodetector is employed such that light coming from a plurality of different assay sites can be detected at a plurality of different sites on the surface of the

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photodetector. The light sources are turned on in sequence so that only a single light source is shining at any one time. The signal detected by the photodetector will correspond to the particular well or spot in the sample beneath the light source which is turned on at that time.

In the preferred embodiment, an array of 96 light-emitting diodes (LED's) is arranged above the 96 wells of a microplate or sample spots on microplate format filter. At least one silicon photodetector is arranged beneath the microplate or filter with an area corresponding to the area of a plurality of the wells on a microplate. Solder connecting lines to the top layer of the photodetector are arranged in a criss-cross fashion to correspond to the area between the sample sites so that light is not blocked.

The use of the large photodetector results in a large noise problem. This noise is overcome by a number of features. By using bright LED's, the intensity of the signal relative to the noise is increased. The primary noise signal encountered is the 60 Hz line power signal. The effect of this noise signal is counteracted by taking 10 readings for each well, with each reading being synchronized to occur 36° after the previous reading in 60 Hz phase space. For 10 readings, the time between readings equals:

$$(\frac{10n + 1}{600}) \quad sec,$$

where n is an integer.

Thus, 60 Hz noise encountered on one reading should be cancelled out by noise encountered on another

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reading at an opposite phase when the 10 samples are averaged.

Noise is also reduced by the use of high-frequency sequencing of the LED's rather than having the light source constantly on. A filter is then used to eliminate noise which occurs at frequencies above the LED sequencing frequencies. Measurements are made at this high frequency for both an on and an off state for each LED. The off state measurement will give a reference level corresponding to the noise in the system at frequencies below that of the sequencing frequency. Because the off state measurement is done immediately after the on state measurement, the noise level should be approximately the same in both states and cancel out.

A disadvantage with using LED's and a large photodetector is that both have characteristics which drift with temperature. These characteristics can change in just a few minutes. Devices which utilize a tungsten light source and small photodiodes do not have drift problems of the same magnitude. This drift problem is overcome by the following steps. A reading is first taken without the sample being inserted to give a photosignal value corresponding to the light transmission through the airspace between th LED's and the photodetector. A second reading is then taken immediately thereafter of a microplate filled with water (blank plate), buffer or any other base solution in which the chemical reactions are to take place or of a blank filter. This will give a "blank" photosignal level reading corresponding to the light transmission through the blank plate. Immediately prior to each subsequent reading of chemical samples, a second reading through an airspace is taken prior to the reading through the

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chemical sample. The chemical sample reading is then
compared to the "blank" reading and is adjusted for
differences between the two airspace readings. The
differences in the readings through air will be
caused by drift of the detector and LED
characteristics with temperature. The use of
airspace readings eliminates the need to insert a
blank prior to each sample.

Turning now to FIG. 8, there is shown a
perspective view of a presently preferred embodiment
of a multibeam photometer according to the present
invention. A photometer 2 is depicted comprising
circuit board 4, the details of which will be
described below, monolithic photodetector 6, LED
array 8, and drawer-type sample holder 10. A
microplate or sample tray 14 is usually placed in
opaque mask 16 adapted to isolate the sample tray
well walls while allowing light to pass through each
sample well from top to bottom or vice versa. This
assembly is then placed in open receptacle 18 of
sample holder 10. The reader is activated by
returning sample holder 10 to the closed position
which activates a microswitch, indicating that the
sample may be read.

FIG. 9 is a side elevation cutaway view of a
microplate well inserted into mask 16 and placed
between photodetector 6 and LED array 8. Although
not depicted, microplate format filters may be read
by placing the filter in a holder adapted to isolate
each sample site one from another while permitting
light to pass through each individual sample site.

FIG. 10 shows a block diagram of the electronic
control circuitry according to the present
invention. A central processing unit (CPU) 40
controls the operation of the circuit and for each

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LED to be illuminated sends a data signal on data
bus 42 to decoders 44 and 46. The decoded signals
are sent through appropriate lines of busses 48 and
52 to a current driver array 50 and a current sink
array 54, respectively, and through drive bus 56 and
sink bus 60 to activate a particular LED in LED
array 8. Light then passes through sample 14 to
photodetector 6. The signal from photodetector 6
passes through current-to-voltage converter 66,
sample and hold circuit 68, analog-to-digital A/D
converter 70, through data bus 72 and back to CPU 40
for analysis.

CPU 40 can be a standard CPU board having a
microprocessor, random access memory (RAM) and read
only memory (ROM), such as the Microsystems 5323. In
operation, when a particular LED is to be turned on,
a digital code corresponding to that LED is
transmitted along data bus 42 to decoders 44 and
46. Decoder 44 decodes a portion of the data
designating the correct column of the LED to be
illuminated, and activates the appropriate line of
data bus 48 to current driver array 50. Decoder 46
decodes the portion of the data corresponding to the
appropriate row of the LED to be illuminated and
activates a data line of data bus 52 connected to
current sink array 54. Current is then passed
through the appropriate line of current driver
bus 56, through the appropriate diode of 8 x 12 LED
array 8 and then through an activated line of sink
bus 60 to the corresponding current sink in current
sink array 54.

The light from a particular LED in LED array 8
will pass through a well in sample tray 14 and be
detected by photodetector 6. The current generated
in photodetector 6 will be converted into a voltage

level by current-to-voltage converter 66. A sample
and hold circuit 68 will sample this analog voltage
level at a period in time controlled by CPU 40. This
analog value will be converted into a digital value
by A/D converter 70. The digital data is passed along
data bus 72 to CPU 40. Data from CPU 40 can be
passed along to a printer 74.

CPU 40 is controlled by an operating program
which decides when data is to be analyzed, printed or
sent to decoders 44 or 46 to illuminate an LED. A
software clock on a line 76 indicates when an LED is
to be illuminated. The timing of this software clock
relative to a 60 Hz line noise signal may vary
because of the variation in the number of program
steps needed to complete an operation being performed
when the program is interrupted to take a reading.
To avoid this variation, software clock 76 is gated
with a hardware clock 78 in a gate 80. Accordingly,
the absolute time of each LED illumination can be
precisely controlled. The output of gate 80 is
coupled to a clock input of decoders 44 and 46.

Photodetector 6 is a rectangular silicon cell
with N-doped material on the top and P-doped material
on the bottom. The N-doped layer is thin enough to .
allow light to pass through. The natural reverse
bias of the silicon cell due to the diffusion of
holes and electrons is sufficient for purposes of the
present invention and no reverse bias voltage is
added. Due to the resistivity of the N-doped region,
a grid of solder lines criss-crosses the top of the
N-doped layer. The lines are located to be in
between the wells of the microplate so that light
transmitted from the LED's will not be blocked.

Current driver array 50 is supplied with current
by a constant current source 82. The amount of

current can be set to correspond to the desired
amount and intensity of light to be emitted by each
LED.

FIGS. 11A and 11B show the detailed circuitry of
portions of the block diagram of FIG. 10. The
circuitry of FIG. 11B is preferably located on the
same circuit card as the 8 x 12 LED array. The
circuitry of FIG. 11A can be located remotely from
the LED array on a separate card.

Referring to FIG. 11A, a current-to-voltage
(I/V) converter 66 is shown which is adapted for
coupling to the photodetector 6 of FIG. 10. A
resistor 84 and capacitor 86 can be adjusted to
provide high frequency filtering of the signal from
photodetector 6. The output voltage signal of I/V
converter 66 is applied to a sample and hold
circuit 68. The sampling and holding functions of
sample and hold circuit 68 are activated by a trigger
input 86. This input originates from one of the data
lines in bus 88 to CPU 40 of FIG. 10. Output 90 of
sample and hold circuit 68 is provided as an input to
A/D converter 70. The maximum value which A/D
converter 70 can accept is 10 volts. Resistor 84
back in I/V converter 66 can be adjusted so that the
largest signals expected to be detected from the
photodetector 6 will produce a voltage of just under
10 volts.

The digital output of A/D converter 70 appears
on data bus 92. Data bus 92 serves to provide the
digital values from A/D converter 70 to CPU 40, as
well as allowing the passage of data from CPU 40 to
decoders 44 and 46. When the CPU is transmitting
data to decoders 44 and 46, the tristate outputs of
A/D converter 70 will be directed to assume a high
impedance state. The outputs of A/D converter 70 are

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controlled by a chip enable signal 94. The digital
output values from A/D converter 70 consist of two
8-bit bytes. A digital signal on the byte
address/short cycle input A_0 activates first the
first byte and then the second byte on the outputs of
A/D converter 70. Thus, more than one output of A/D
converter 70 is shown coupled to each data line.

A decoder 96 provides an enable signal 98 to
decoders 44 and 46 of FIG. 11B. This signal also
serves as the software clock input to synchronizing
circuit 80. The output of synchronizing circuit 80
is an inhibit signal 100.

Referring to FIG. 11B, data signals D0-D6 are
shown input to decoders 44 and 46. A constant
current source 82 provides current for current
drivers 50 to LED array 58. The rows of LED array 8
are coupled to current sinks 54, which are inverters
having an open collector output.

When a particular LED is selected, a 7-bit
digital code will appear on data lines D0-D6. When
an enable signal is provided and the inhibit signal
is removed, decoder 44 will activate one output line
and decoder 46 will activate one output line. The
line activated by decoder 44 will switch one of
current drive transistors 50 which will provide
current from current source 82 to all of the LED's in
one of columns A-H. The output line activated by
decoder 46 will turn on one of inverters 54 to a low
state output, thereby coupling the selected one of
rows 1-12 to ground. This will allow current to flow
through the selected one of the LED's in the selected
one of rows A-H.

A front panel display 12 in FIG. 11A has
"ready," "load," and power on indicator lights as
well as "set blank," "read tray," and "optical

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density (OD)/range" switches. The switches and lights are controlled by signals from the CPU as discussed below.

The operation of the circuit of FIGS. 10 and 11 can be understood with reference to the timing diagram of FIG. 12. The sample and hold trigger signal is signal 86 of FIG. 11A, the decoder inhibit is signal 100 of FIG. 11A and FIG. 11B, and the A/D chip enable signal is signal 94 of FIG. 11A. Two separate readings are taken for each sample, one with the LED on and one with the LED off. The transmittance level is the difference between the on and off readings. The sequences of the on and off readings are shown separately. All 96 LED's are activated in sequence, and the process is repeated 10 times over equally spaced phase positions of a 60 Hz line noise signal.

The sample and hold trigger signal on line 86 produces a pulse every 250 microseconds (ms). This pulse pulse is generated in the CPU with the hardware clock from a crystal oscillator. The decoder inhibit signal is produced by a combination of the hardware clock and a software clock. The software clock signal is produced as soon after an interrupt signal in the CPU program as the operation of the CPU program can be interrupted . This amount of time can vary from 4 to 20 clock cycles, depending upon the status of program operation. When such a software clock signal is produced on line 98, it is combined with the hardware clock in synchronizing circuit 80 to produce a down going pulse on the decoder inhibit signal at time 104. This causes a particular LED to be turned on as explained before.

Sample and hold circuit 68 is enabled for sampling a value at time 106. This sample is locked

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in at a hold time 108. This hold time is delayed
past the LED turn-on time 104 to allow the signal to
be processed through current-to-voltage converter 66
and filtered through resistor 84 and capacitor 86.
A/D converter 70 has its tristate outputs enabled at
a point of time 110 and data is read out in two bytes
under the control of two chip enable pulses 112.

Similarly, a sequence for recording a sample
when the LED is turned off begins at a point of
time 114 when the inhibit signal again appears,
thereby disabling the decoders and removing current
from the current drivers to turn off the LED's.
Sample, hold, start of A/D and read of A/D signals
follow accordingly.

Ten on and off readings of this type are taken
for each LED with all 96 LED's being sequenced for
the first on and off reading, then all 96 being
sequenced for the second on and off reading, and so
forth. Because the most predominant noise signal is
the 60 Hz line voltage, these 10 readings are equally
spaced along different positions of the phase of the
60 Hz cycle. Thus, a first reading may be taken
along the rising edge of the first 60 Hz cycle, and
after the remaining 95 LED's are sequenced, a second
reading is taken at a higher point on a corresponding
later 60 Hz cycle. Because of the time difference
between an LED on and LED off reading the ground or
reference voltage, which is actually changed by the
60 Hz noise signal, will move slightly, thereby
causing an error. This movement will be slightly
upward for a reading taken on one part of a 60 Hz
cycle, and slightly downward for a reading taken
during another portion of a 60 Hz cycle. Thus, by
equally spacing the readings across the 60 Hz cycle,
the errors in the 10 readings should cancel out when
an average value is taken.

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Prior to insertion of a microplate, which is to be read or used as a blank plate, drawer 10 is pulled out and the plate reader will do a reading of the airspace in between the LED's and the photodetector and after completion will light up the "load" indicator in front panel 12. The reading of microplates containing experimental samples is preceded by the insertion of a microplate containing a reference fluid such as water (or a set of reactants at time equals zero (t = 0) to give a "blank," or t = 0 reading. The "ready" indicator will light up when the tray is all the way in and the instrument is capable of responding to the "set blank" or "read" switch. The readout on printer 74 can be either several digits of resolution or can be rounded to a single digit as controlled by the "optical density (OD) range" switch.

After the blank value has been determined and drawer 10 pulled out fully, an experimental sample tray can be placed in the drawer. The plate reader will again measure the airspace between the LED's and the photodetector immediately prior to reinsertion of drawer 10. The photosignal values as modified by the various wells of the sample tray are compared to values for the blank tray and are adjusted for variations in the airspace reading. Thus, a blank tray can be inserted initially and will not have to be reinserted for sample readings taken over the course of several hours or days. The adjustments for drift in the LED's and the photodetector with temperature over this time period is made by the comparison of the two airspace readings immediately before the blank tray insertion and immediately before the particular sample tray insertion.

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The on-board software program which controls the
operation of the microplate reader is shown in the
flow chart of FIG. 13. The plate reader is first
turned on and housekeeping functions are performed by
the software (step A). The operator then pulls
drawer 10 out. The "drawer out" state is then
detected (step B) and the LED's are scanned (step C)
to produce a series of photosignal values (step D)
corresponding to light transmission through the·
airspace. These values are put in a Table 3 in
memory.

Once drawer 10 has been reinserted (step E), the
front panel switches are checked to see whether the
drawer contains a blank tray (step F). For a blank
tray, the LED's are scanned (step G) and the
photosignal values are put in a Table 1 in memory
(step H). The airspace values of Table 3 are then
transferred to another Table 2 (step I) to provide a
set of airspace readings close to the time of the
blank plate reading producing the values of
Table 1. The airspace readings are done constantly
when drawer 10 is out, thus constantly updating
Table 3 prior to drawer 10 containing a tray being
inserted.

If drawer 10 is inserted and the "set blank"
switch is not thrown, the front panel switches are
examined to determine whether the samples are to be
red (step J). If the "read" switch has been thrown,
the LED's are scanned (step K) and the transmittance
values are put in Table 4 in memory (step L). The
optical densities for the samples are then calculated
(step M) and printed (step N).

The calculation of optical density (OD) is done
in accordance with the following equation:

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$$OD_n = \log (n_1)(n_3)/(n_4)(n_2).$$

where the photosignal value of the nth
site in Table X equals n_X; and

the optical density of the sample at
site n equals OD_n.

Each LED is scanned starting with the first and
proceeding through the 96th as discussed earlier.
Ten repetitions are done equally spaced over the
phases of a 60 Hz cycle. The final photosignal
values used in the calculation described above are
given by the sum of the 10 or minus off signals. As
can be seen by the above equation, the optical
density is the log of the blank value divided by the
sample value adjusted for differences in the airspace
readings.

A photosignal reading in accordance with the
flow chart of FIG. 13 is initiated by an interrupt
signal to a microprocessor in CPU 40. In between
readings initiated by the interrupt signal, the
program in the CPU can cause data to be calculated or
transferred to one of the tables or printed out on a
printer. The actual reading itself is sandwiched in
between these other functions as dictated by the
interrupt signal. Because the interrupt signal will
be interrupting a program operation, a certain amount
of steps after the interrupt signal are necessary to
basically hold the place in the program by moving
data into memory or completing a step or otherwise.
Thus, the amount of time between the initiation of an
interrupt signal and the reading of an LED could vary
(usually by between 4 and 20 clock cycles). To
prevent this from causing jitter in the readings, the

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interrupt is provided at least 20 clock cycles prior
to when it is anticipated that a reading should be
taken and is, in effect, ANDed with a real time clock
in synchronizing gate 80 as shown in FIGS. 3 and 4.
This ensures that the location of each reading
relative to the phase of the 60 Hz noise signal will
be precisely controlled.

Rather than manually flicking the switches of
control panel 12, the operation of the plate reader
can be controlled by another computer, such as a
microcomputer coupled to the plate reader. The
attachment of such a host computer overrides the
operation of the switches.

The rapid solid state multibeam photometer as
described above is characterized by its ability to
reliably read transmittances of multi-site solid
support assay samples in less than about 2 seconds
without movement of the sample or holder. This is
achieved by employing at least one monolithic
photodetector of an area at least equal to the area
of a plurality of multi-site solid support assay
sites.

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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A multibeam photometric measurement device for measuring optical density of samples contained in a plurality of sample sites disposed on a multi-site assay plate, said device comprising:

a single light source adapted through coupling means to be selectively applied, according to a predetermined sequence through selected cones of a plurality of light transmitting means to selected ones of said samples sites;

a plurality of photodetector means disposed on a detector board and positioned in relation to said assay plate in such a way that light transmitted through each of said sites is received by a corresponding photodetector;

means for simultaneously imparting mixture movement to said samples comprising means for imparting movement to said assay plate for a predetermined time before a reading is performed on a sample site; and

means for analyzing the output of said photodetector means to determine and indicate the optical density of said sites.

2. As measurement device as in claim 1, wherein said light source provides ultraviolet light.

3. A measurement device as in claim 2 which further comprises a spectral balancing filter to filter light from said light means and provide light of substantially constant intensity over the visible and ultraviolet spectrum.

4. A measurement device as in claim 1 wherein said light transmitting means comprise optical transmission fibers and said coupling means comprises:

a fiber rotor including a rotor optical fiber receiving substantially all of said light at an input end transmitting it to an output end; and

a fiber distributor including said plurality of transmission optical fibers disposed in a manner allowing each of said transmission optical fibers to be selectively coupled to the light coming out of the output end of said rotor fiber;

said fiber rotor capable of being displaced in such a way that said optical fiber couples light from said source to successive ones of said transmission optical fibers and hence to successive ones of said sample sites.

5. A measurement device as in claim 4 which further comprises:

means for modulating the light source at a predetermined rate; and

means for filtering light of a desired wavelength from said light source for application to said sample sites.

6. A measurement device as in claims 4 or 5 wherein said fibers comprise material which emits substantially no light of wavelengths other than that which it receives.

7. A measurement device as in claim 4 wherein said distributor also contains a first reference optical fiber directly coupling said light to a first reference photodetector on said detector board.

8. A measurement device as in claim 7 wherein said first reference fiber functions as a means for referencing the relative positioning of said fiber rotor with respect to said distributor.

9. A measurement device as in claim 7 wherein said first reference fiber serves as a light referencing means.

10. A measurement device as in claim 8 wherein said first reference fiber also serves as a light referencing means.

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11. A measurement device as in claim 6 further including a second reference optical fiber directly coupling said light to a second reference photodetector disposed on said detector board and functioning as light referencing means.

12. A measurement device as in claim 7 wherein said assay plate comprises 96 sample sites disposed in an 8x12 matrix and said detector board comprises a corresponding 8x12 matrix of photodetectors in addition to a single photodetector corresponding to first reference fiber.

13. The apparatus of claim 4 wherein said means for analyzing comprises:

means for sequentially selecting the analog output signals of desired photodetectors for analysis;

means for converting said analog signals to their corresponding digital values;

means for amplifying said analog signals in such a way as to utilize a substantial portion of the dynamic range of said analog to digital conversion means;

means for storing precalculated values corresponding to said digitized values, accepting said digitized values and retrieving the corresponding stored precalculated value in response to each of said digital values; and

means for processing said retrieved values in order to calculate the optical density of said samples.

14. A measurement device as in claim 13 wherein said photodetectors are divided into blocks, each consisting of a plurality of photodetectors, in such a way that photodetectors in said blocks are spaced substantially apart on said detector board, and the outputs of the photodetectors of each said block are connected together to yield a common output signal, whereby the output of a selected photodetector can be analyzed by selecting the common output signal for the block in which the photodetector lies.

15. A measurement device as in claim 14 wherein each of said blocks comprises 6 photodetectors.

16. A measurement device as in claim 13 wherein said means for analyzing further comprises means for analyzing that portion of said analog signal which is modulated at said predetermined rate.

17. A measurement device as in claim 14 wherein the distributor further comprises means for preventing light from being coupled into said light transmitting means and said means for analyzing includes means for measuring any output from the analog to digital conversion means in the absence of light being coupled to the transmitting means.

18. A measurement device as in claim 1 which further comprises a drive motor for advancing and retracting said assay plate relative to said detectorboard.

19. A measurement device as in claim 18 wherein said drive means further serves for imparting said movement to said assay plate.

20. A photometric measurement device for measuring optical density of samples contained in a plurality of sample sites disposed on a multi-site assay plate, said device comprising:

a light source for applying light to selected ones of said sample sites;

photodetector means positioned in relation to said assay plate in such a way that light transmitted through each of said sites is received by said photodetector;

means for simultaneously imparting mixing movement to said samples comprising means for imparting movement to said assay plate for a predetermined time before a reading is performed on a sample site; and

means for analyzing the output of said photodetector

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means to determine and indicate the optical density of said sites, said means for analyzing comprising:

means for sequentially selecting the analog output signals of desired photodetectors for analysis;

means for converting said analog signals to their corresponding digital values;

means for amplifying said analog signals in such a way as to utilize a substantial portion of the dynamic range of said analog to digital conversion means;

means for storing precalculated values corresponding to said digitized values, accepting said digitized values and retrieving the corresponding stored precalculated value in response to each of said digital values; and

means for processing said retrieved values in order to calculate the optical density of said samples.

21. A measurement system as in claim 20 wherein said means for imparting mixing movement to said samples comprises means for imparting movement to said assay plate.

22. A measurement device as in claims 20, wherein said light source provides ultraviolet light.

23. A measurement device as in claim 20 wherein said means for analyzing further comprises means for analyzing that portion of said analog signal which is modulated at a predetermined rate.

24. A photometric measurement device for measuring optical density of samples contained in a plurality of sample sites disposed on a multi-site assay plate, said device comprising:

a light source for applying light to selected ones of said sample sites;

photodetector means positioned in relation to said assay plate in such a way that light transmitted through each of said sites is received by said photodetector;

means for simultaneously imparting mixing movement
to said samples comprising means for predetermined time
before a reading is performed on a sample site; and

means for analyzing the output of said
photodetector means to determine and indicate the optical
density of said sites,

wherein said photodetectors are divided into
blocks, each consisting of a plurality of photodetectors, in
such a way that photodetectors in said blocks are spaced
substantially apart on said detector board, and the outputs
of the photodetectors of each said block are connected
together to yield a common output signal, whereby the output
of a selected photodetector can be analyzed by selecting the
common output signal for the block in which the photodetector
lies.

25. A measurement system as in claim 24 wherein said
means for imparting mixing movement to said samples comprise
means for imparting movement to said assay plate.

26. A measurement device as in claims 24, or 25 wherein
said light source provides ultraviolet light.

27. A measurement device as in claim 24 wherein each of
said blocks comprises 6 photodetectors.

28. A measurement device as in claim 24 wherein said
means for analyzing further comprises means for analyzing
that portion of said analog signal which is modulated at a
predetermined rate.

29. A measurement device as in claims 13 or 20 wherein
said means for amplifying comprises:

a amplifier means for receiving said analog signals
and providing an amplified output signal;

means for comparing the digital value provided by
said analog to digital converter means in response to said

amplified output signal with the maximum possible value of said analog to digital converter means; and

means for adjusting the gain possible without exceeding the maximum possible value of said analog to digital converter means.

30. A measurement device as in claim 29 wherein said amplifier means comprises:

a first amplifier having a gain set in response to a light signal in the absence of a sample; and

a second amplifier having a gain set in response to a light signal passing through a sample.

31. A photometric kinetic measurement device for analyzing samples contained in a plurality of sample sites disposed on a multi-site assay plate, the device comprising:

a light source for providing light to one or more of said sample sites;

photodetector means for receiving light transmitted through each of said one or more sample sites and for providing a signal in response to said light received wherein the light vertically passes through the sample;

means for imparting oscillatory movement to said multi-site assay plate for a pre-determined time before each of a series of readings is performed on a sample site in order to agitate the sample therein; and

a means for kinetic analysis of the signal of the photodetector means.

32. A method for measuring the transmittance of a plurality of sample sites on a multi-site assay plate comprising the steps of:

selectively focusing light from a single light source through a plurality of light transmitting means upon selected ones of said sample sites,

for each sample site, measuring, at a succession of time intervals, the intensity of light passing through the

reacting sample in the sample site by using photodetecting means,

oscillating said assay plate for a first fixed time duration in order to agitate the samples contained therein and allowing the agitated samples to settle for a second fixed time duration before performing each of said successive measurements,

measuring the intensity of light when coupled directly from said light transmitting means to said photodetecting means,

measuring the intensity of light when coupled from said light transmitting means to said photodetecting means, through the sample site when it contains no reacting sample,

using said measurements to calculate the optical density of said sample sites.

33. A method for taking measurements of the transmittance of a plurality of sample sites on a multi-site assay support, comprising the steps of:

measuring the photosignal generated by light shining through an airspace in the absence of a sample to give a first air transmission photosignal value;

measuring the photosignal generated by light shining through an airspace in the absence of said support to give a second air transmission photosignal value;

adjusting said relationship to compensate for a difference between said first air photosignal value and said second air photosignal value.

34. A method for determining a colorimetric assay utilizing a multibeam photometer having a photodetector means positioned in relation to a multi-site solid such that light is received at a plurality of different sites on the surface of said photodetector means, said method comprising the following steps:

measuring the photosignal generated by light shining through an airspace in the absence of a sample support to give a first air transmission photosignal value;

measuring the photosignal generated by light shining
through an airspace in he absence of said support to give a
second air transmission photosignal value;

adjusting said relationship to compensate for a
difference between said first air photosignal value and said
second air photosignal value.

35. A method for measuring the transmittance of a
plurality of samples contained in a plurality of sample sites
on a multi-site assay plate while said assay plate is in a
device for carrying out said measuring, said method
comprising the steps of:

      inserting said assay plate into said device;

      imparting mixing movement to said samples by
imparting movement to said assay plate for a first
fixed time duration;

      providing light to one or more of said sample
sites;

      for each sample site, measuring the intensity of
light passing through the sample in the sample
site; and

      removing said assay plate from said device.

36. A method for measuring the transmittance of a
plurality of samples contained in a plurality of sample sites
on a multi-site assay plate while said assay plate is in a
device for carrying out said measuring, said method
comprising the steps of:

    (a)   inserting said assay plate into said device;

    (b)   sequentially, for each said sample, performing
the steps of:

        (1)   imparting mixing movement to said sample
by imparting movement to said assay plate
for a first fixed time duration;

        (2)   providing light to one or more of said
sample sites; and

        (3)   measuring the intensity of light passing
through said sample; and

(c)   removing said assay plate from said device.

37.   A method as in Claim 35 which further includes the step of, following said step of imparting mixing movement to said sample, waiting for a second fixed time duration prior to said step of measuring.

38.   A method as in Claim 36 which further includes the step of, following said step of imparting mixing movement to said sample, waiting for a second fixed time duration prior to said step of measuring.

39.   A method as in Claim 36 which further comprises the step of, prior to step (c), repeating step (b).

40.   A method as in Claim 39 which further comprises the steps of:
    i)   measuring the passage of time between said measuring steps, for each said sample; and
    ii) calculating the rate of change of light transmission or optical density of each said sample.

41.   A method for measuring the transmittance of a plurality of samples contained in a plurality of sample sites on a multi-site assay plate while said assay plate is in a device for carrying out said measuring, said method comprising the steps of:
    (a)   inserting said assay plate into said device;
    (b)   imparting mixing movement to said samples by imparting movement to said assay plate for a first fixed time duration;
    (c)   providing light to one or more of said sample sites;
    (d)   for each said sample, measuring the intensity of light passing through the sample in the sample site by using photodetecting means; and
    (e)   removing said assay plate from said device.

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42. A method as in Claim 41 which further includes the step of, following said step of imparting mixing movement to said samples, waiting for a second fixed time duration prior to said step of measuring.

43. A method as in Claim 42 wherein said step of measuring comprises the step of measuring each sample sequentially.

44. A method as in Claim 40 which further comprises the step of, prior to step (e), repeating steps (b), (c), and (d).

45. A method as in Claim 44 which further comprises the steps of:

    i)   measuring the passage of time between said measuring steps, for each said sample; and

    ii) calculating the rate of change of light transmission or optical density of each said sample.

46. The method as in Claim 36 wherein said steps of inserting and removing include the step of aligning said assay plate within said device with respect to said photodetector means.

47. The method as in Claim 40 wherein said steps of inserting and removing include the step of aligning said assay plate within said device with respect to said photodetector means.

48. The method as in Claim 46 wherein said steps of aligning and imparting movement are performed utilizing a common drive mechanism.

49. The method as in Claim 47 wherein said steps of

- 62 -

aligning and imparting movement are performed utilizing a common drive mechanism.

50. A method as in Claim 36 which further comprises the steps of:

measuring the intensity of light when coupled directly to said photodetecting means; and

using said measurements to calculate the optical density of said sample sites.

51. A method as in Claim 41 which further comprises the steps of:

measuring the intensity of light when coupled directly to said photodetecting means; and

using said measurements to calculate the optical density of said sample sites.

52. A method as in Claims 37 or 44 which further comprises the steps of:

measuring the intensity of light when coupled directly to said photodetecting means;

using said measurements to calculate the optical density of each said sample at a plurality of times; and

using the optical density of each said sample at a plurality of times to determine the rate of change of optical density of each said sample.
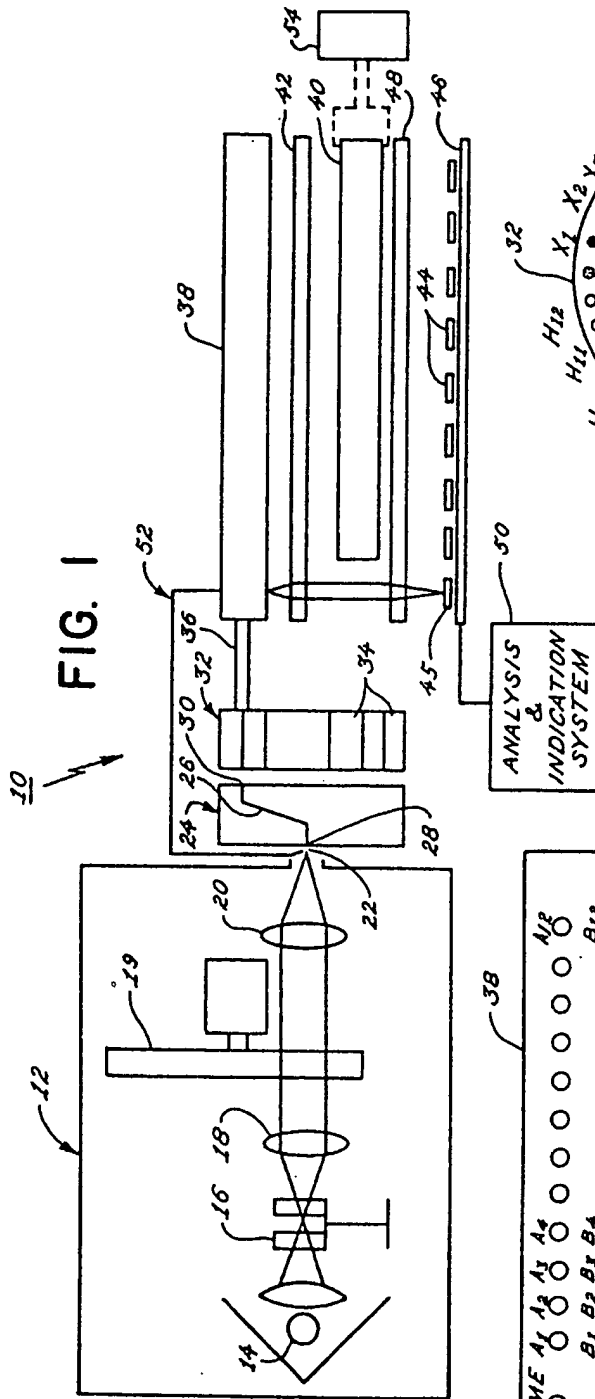
FIG. 1

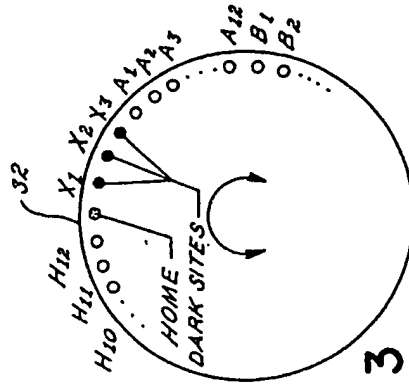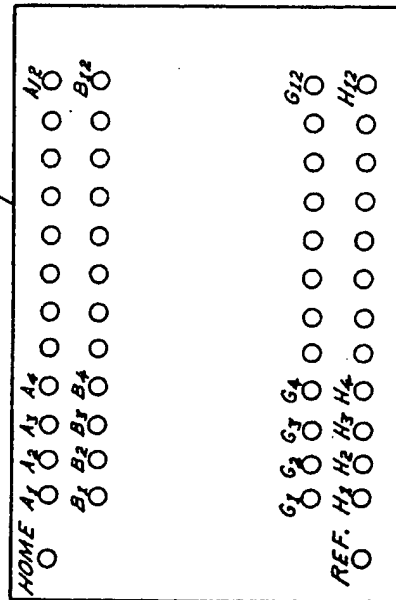FIG. 2

FIG. 3

HOME

DARK SITES

X_1 X_2 X_3 A_1 A_2 A_3 A_{12} B_1 B_2

H_{12} H_{11} H_{10}

ANALYSIS & INDICATION SYSTEM

HOME A_1 A_2 A_3 A_4 A_{12}

B_1 B_2 B_3 B_4 B_{12}

G_1 G_2 G_3 G_4 G_{12}

REF. H_1 H_2 H_3 H_4 H_{13}

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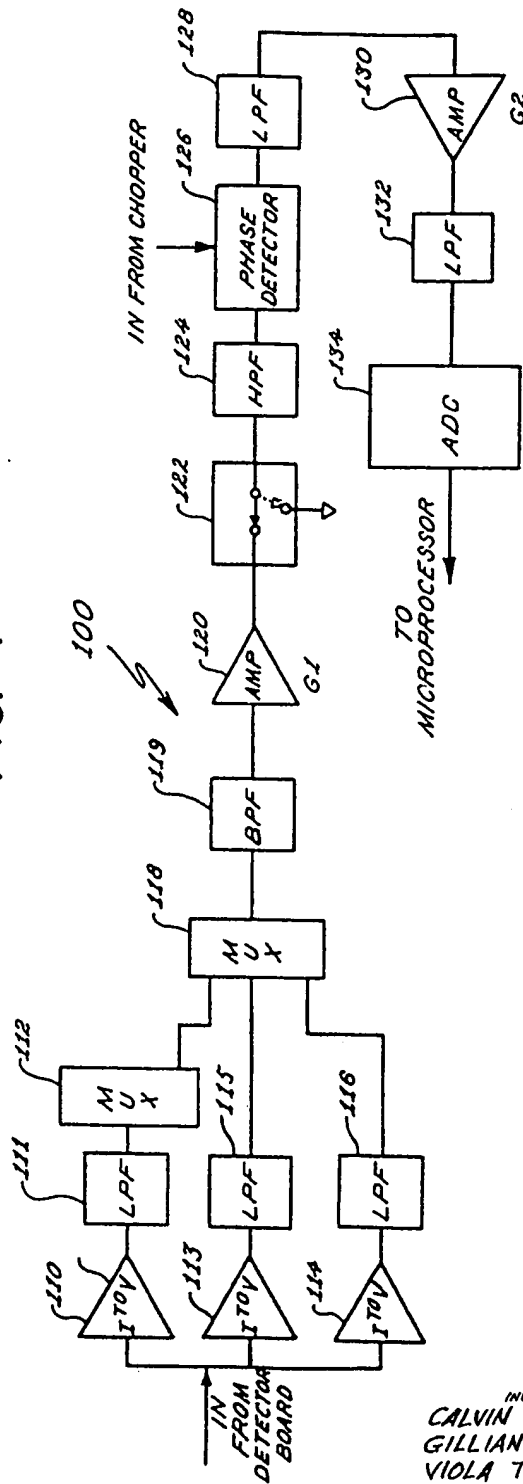Fetherstonhaugh & Co.

FIG. 4

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FIG. 5

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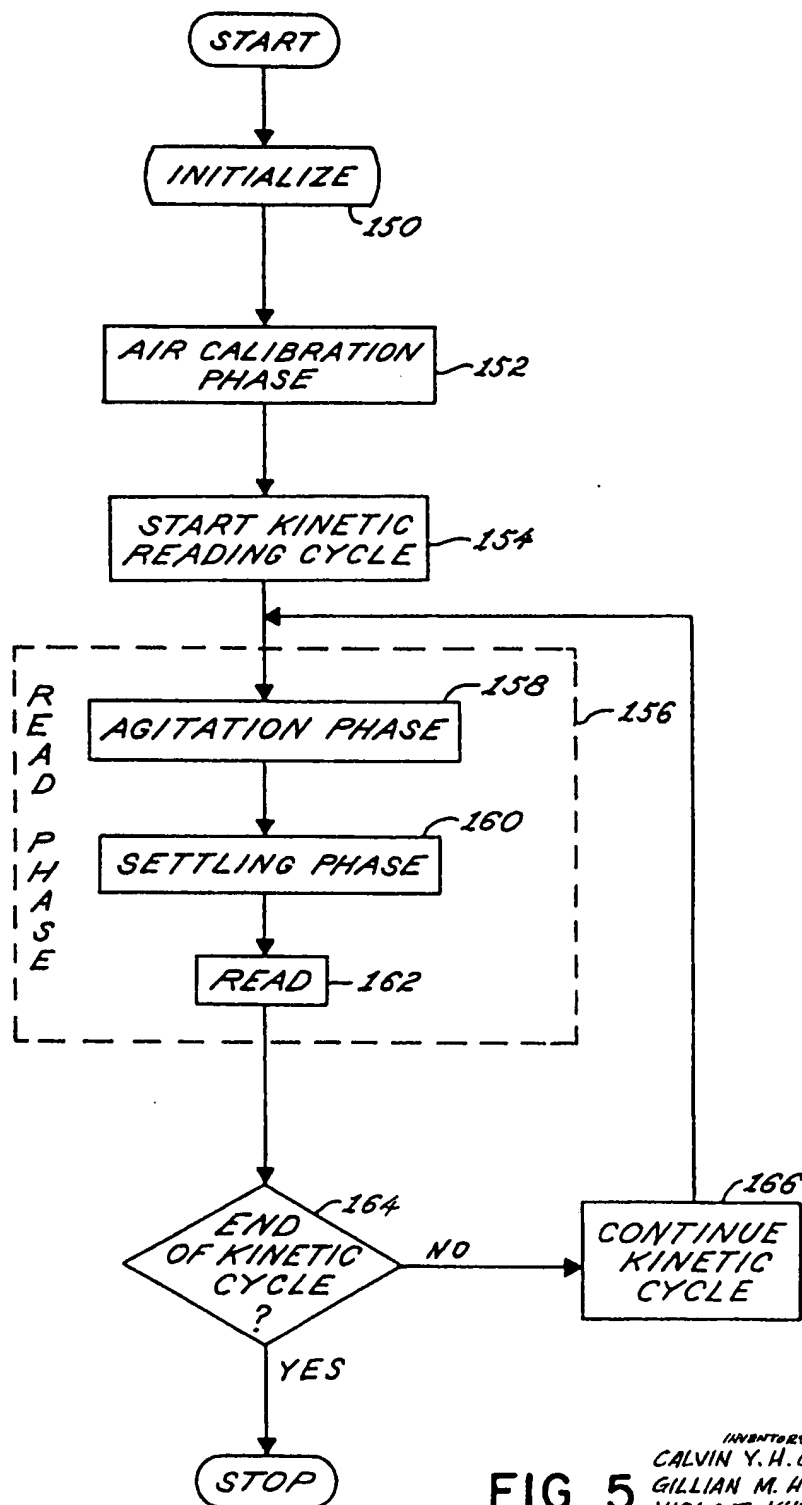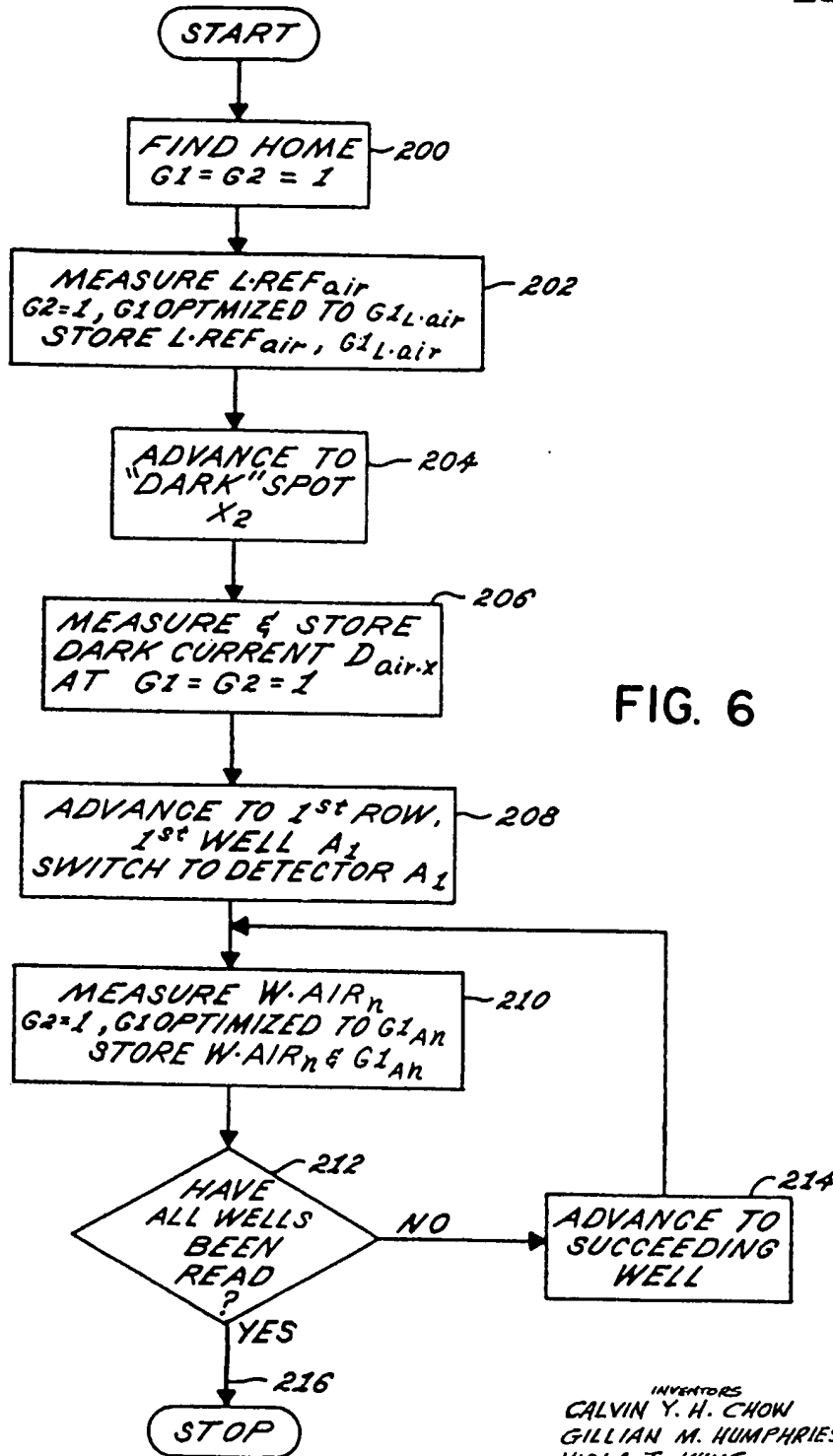
START

FIND HOME
$G1 = G2 = 1$ — 200

MEASURE $L \cdot REF_{air}$
$G2=1, G1$ OPTMIZED TO $G1_{L \cdot air}$
STORE $L \cdot REF_{air}$, $G1_{L \cdot air}$ — 202

ADVANCE TO
"DARK" SPOT
X_2 — 204

MEASURE & STORE
DARK CURRENT $D_{air \cdot x}$
AT $G1 = G2 = 1$ — 206

FIG. 6

ADVANCE TO 1st ROW,
1st WELL A_1
SWITCH TO DETECTOR A_1 — 208

MEASURE $W \cdot AIR_n$
$G2=1, G1$ OPTIMIZED TO $G1_{An}$
STORE $W \cdot AIR_n$ & $G1_{Ah}$ — 210

HAVE
ALL WELLS
BEEN
READ
? — 212

NO → ADVANCE TO
SUCCEEDING
WELL — 214

YES — 216

STOP

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START

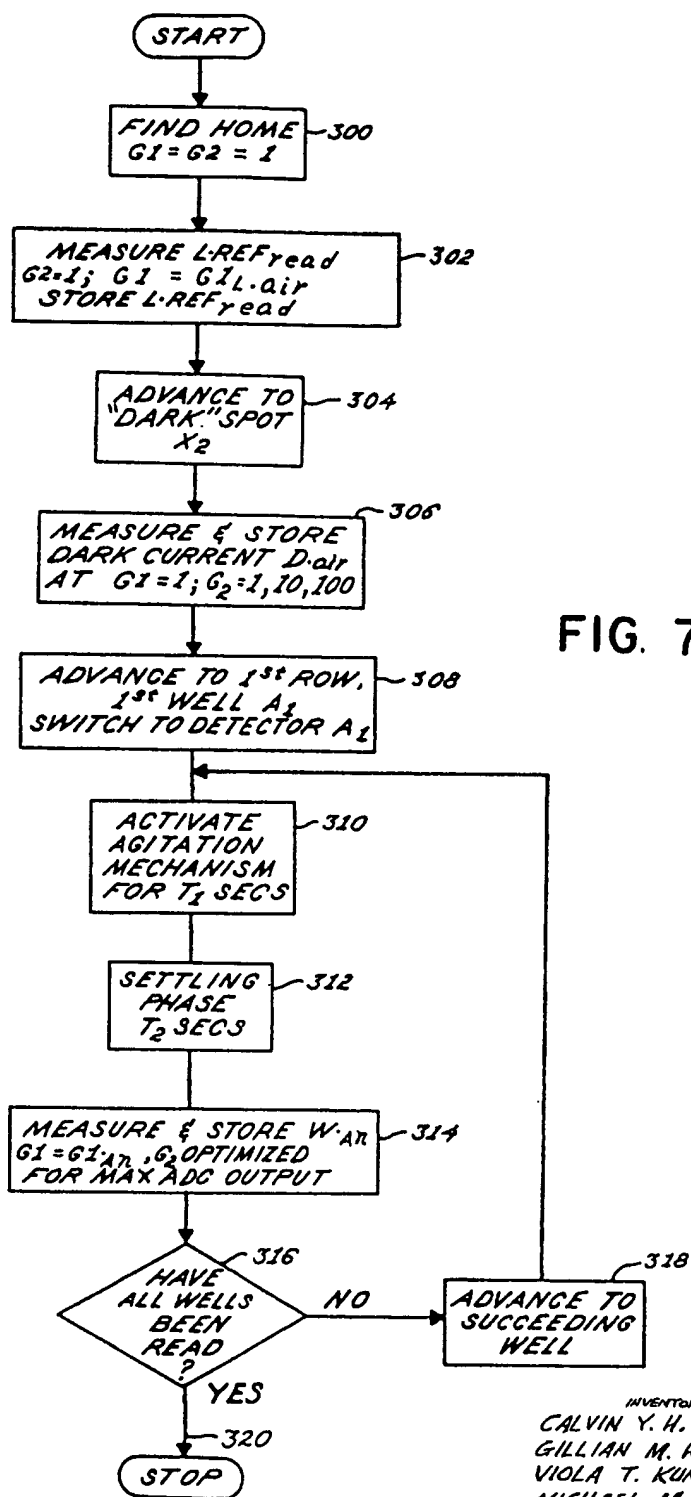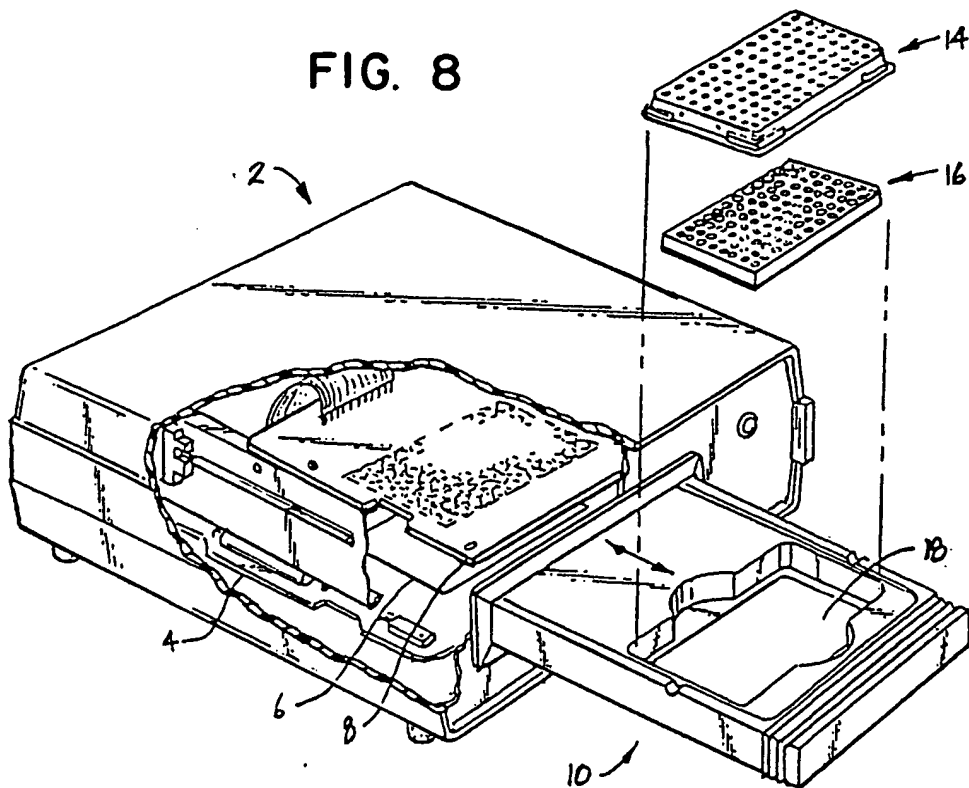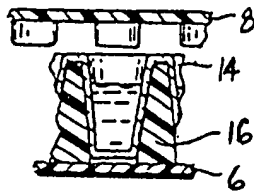| FIND HOME
$G1 = G2 = 1$ | —300 |

| MEASURE $L \cdot REF_{read}$
$G2 = 1$; $G1 = G1_{L \cdot air}$
STORE $L \cdot REF_{read}$ | —302 |

| ADVANCE TO
"DARK" SPOT
X_2 | —304 |

| MEASURE & STORE
DARK CURRENT $D \cdot air$
AT $G1 = 1$; $G_2 = 1, 10, 100$ | —306 |

| ADVANCE TO 1st ROW,
1st WELL A_1
SWITCH TO DETECTOR A_1 | —308 |

FIG. 7

| ACTIVATE
AGITATION
MECHANISM
FOR T_1 SECS | —310 |

| SETTLING
PHASE
T_2 SECS | —312 |

| MEASURE & STORE $W \cdot A_n$
$G1 = G1 \cdot A_n$, G_2 OPTIMIZED
FOR MAX ADC OUTPUT | —314 |

HAVE
ALL WELLS
BEEN
READ
? —316

NO → ADVANCE TO
SUCCEEDING
WELL —318

YES

STOP —320

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FIG. 8

FIG. 9

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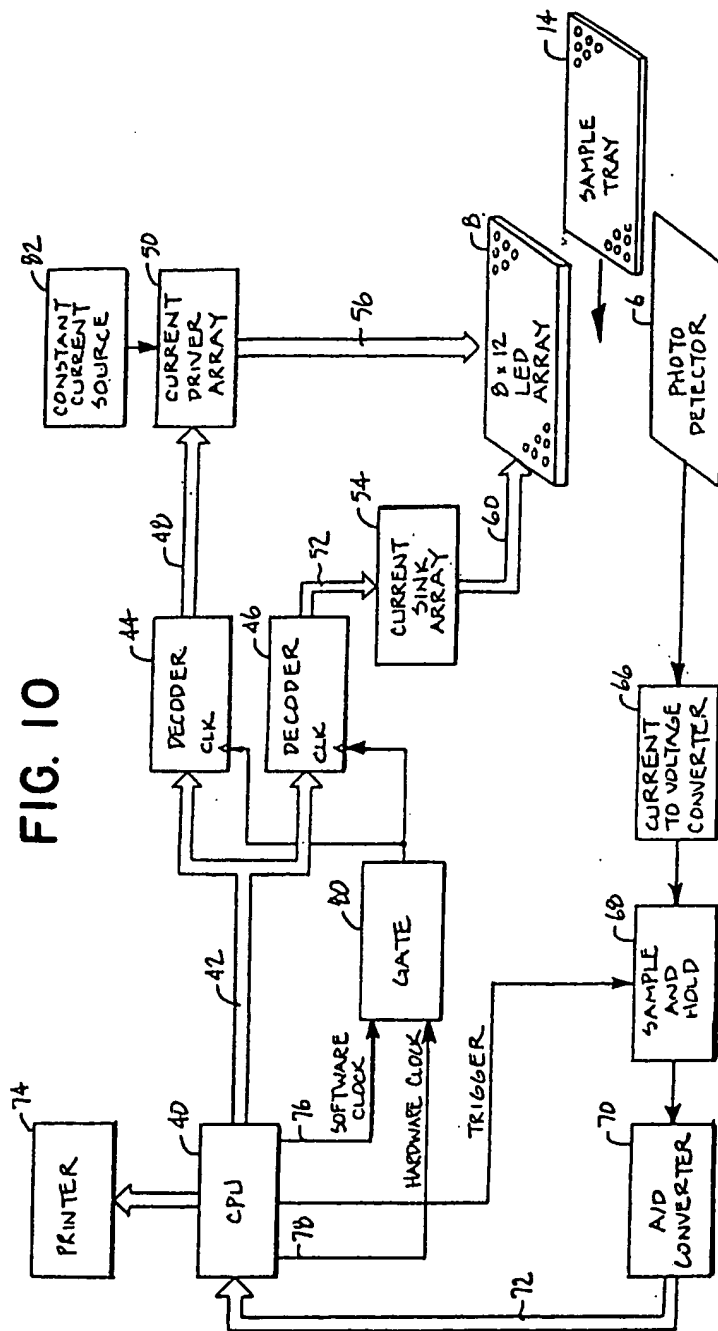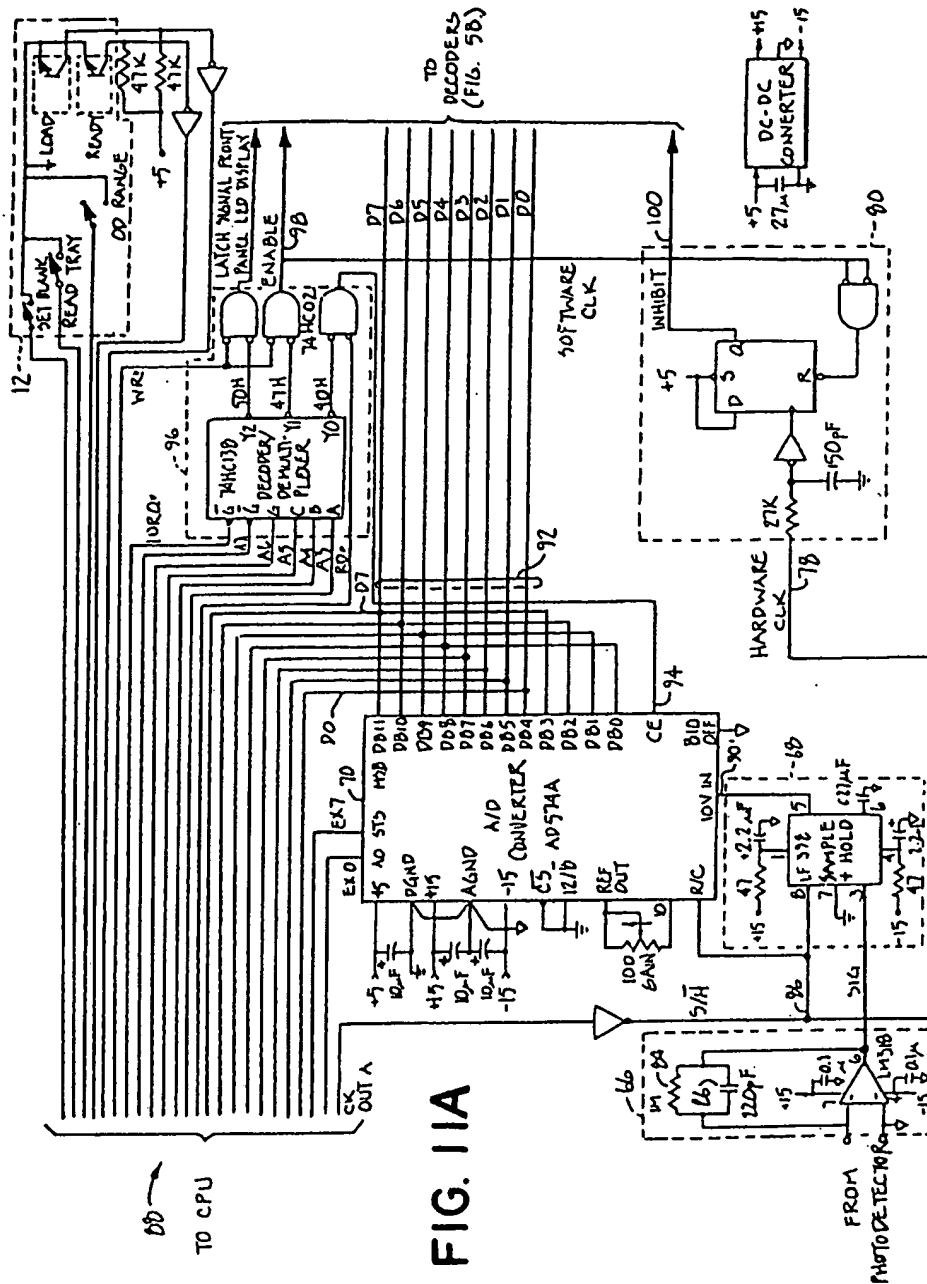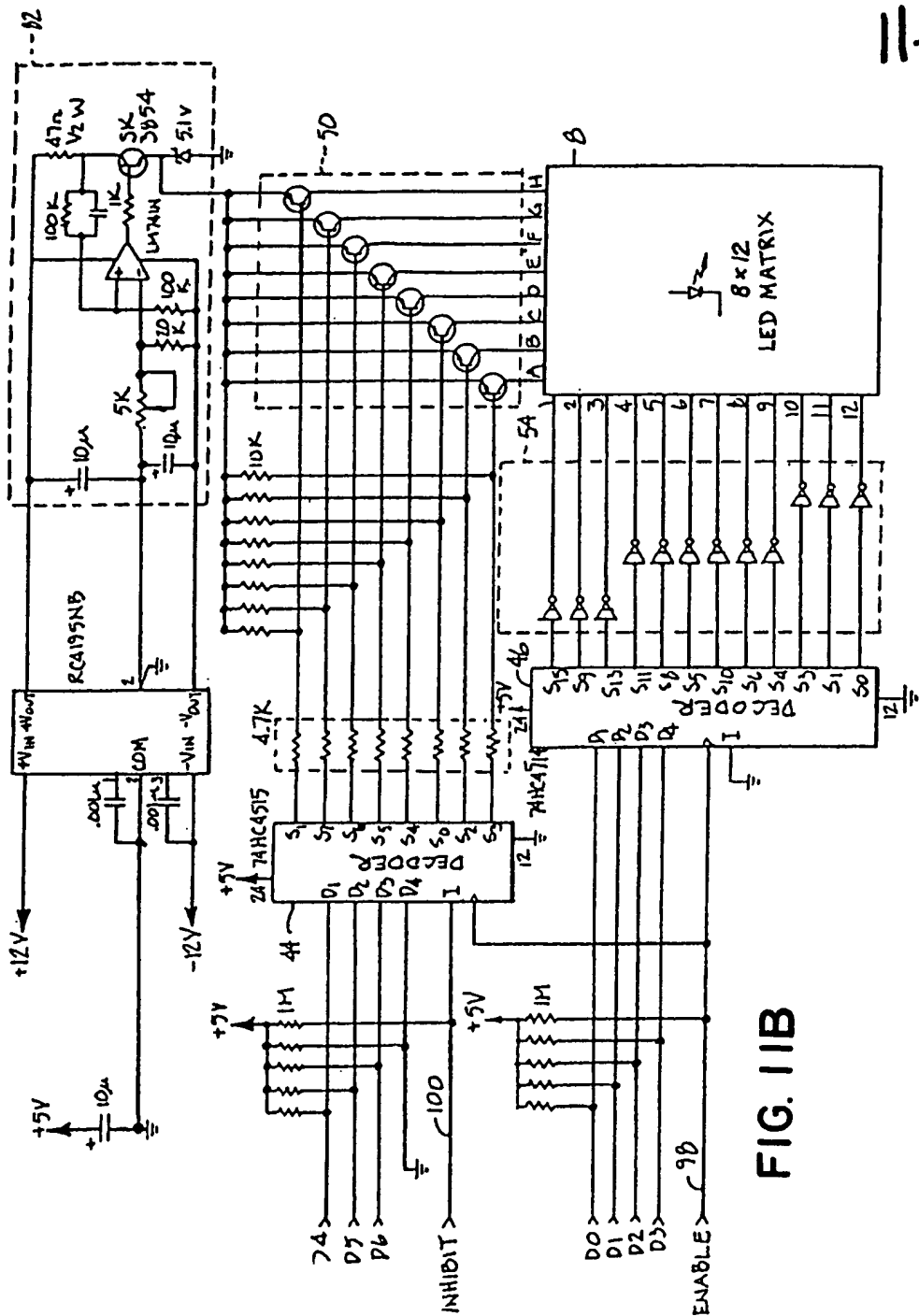MICHAEL M. LACY
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FIG. 10

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FIG. IIA

TO CPU

TO DECODERS (FIG. 5B)

DC-DC CONVERTER

INHIBIT

SOFTWARE CLK

HARDWARE CLK

A/D CONVERTER AD574A

SAMPLE + HOLD

FROM PHOTODETECTOR

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FIG. 11B

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FIG. 12

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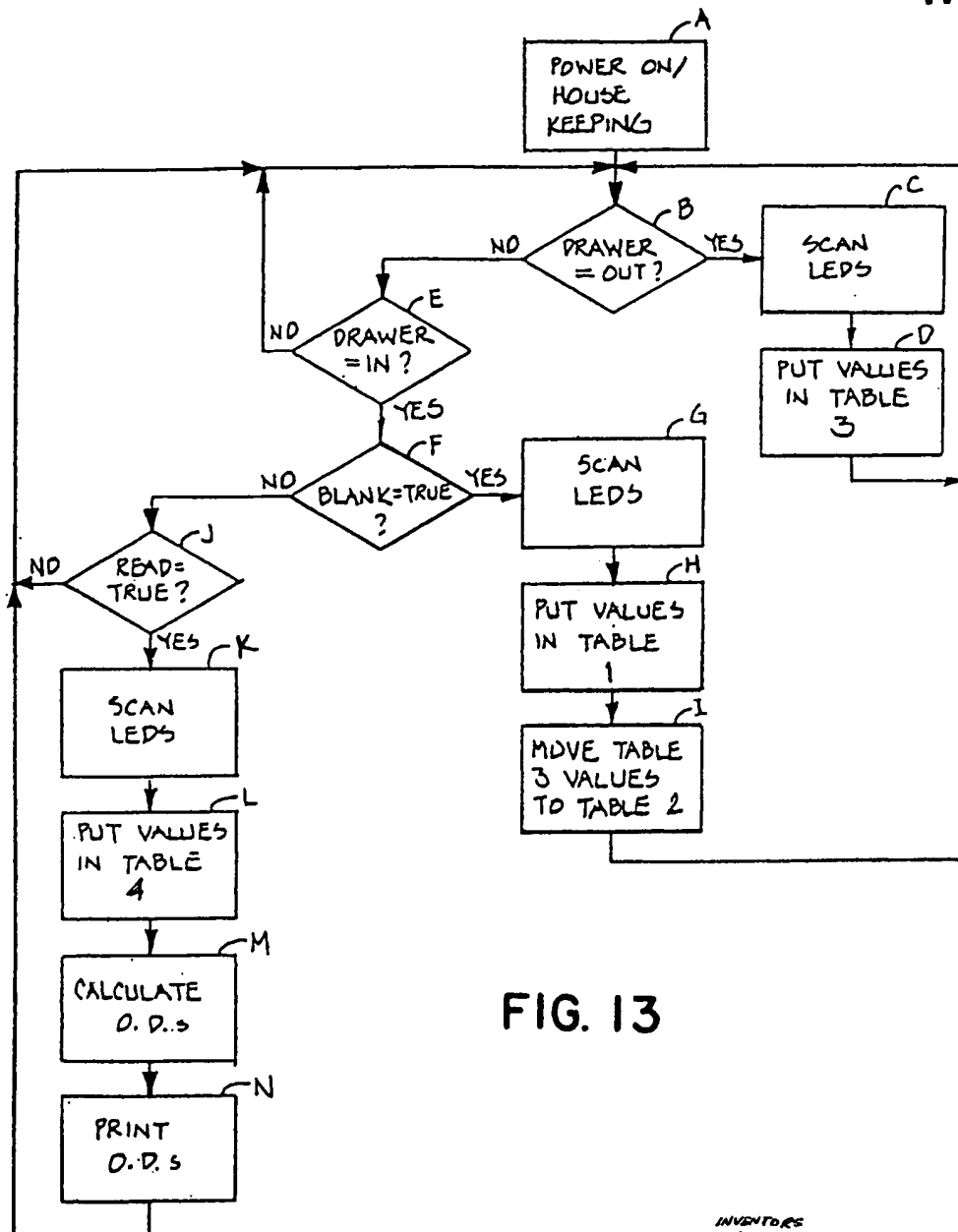JOHN W. PARCE
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FIG. 13

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